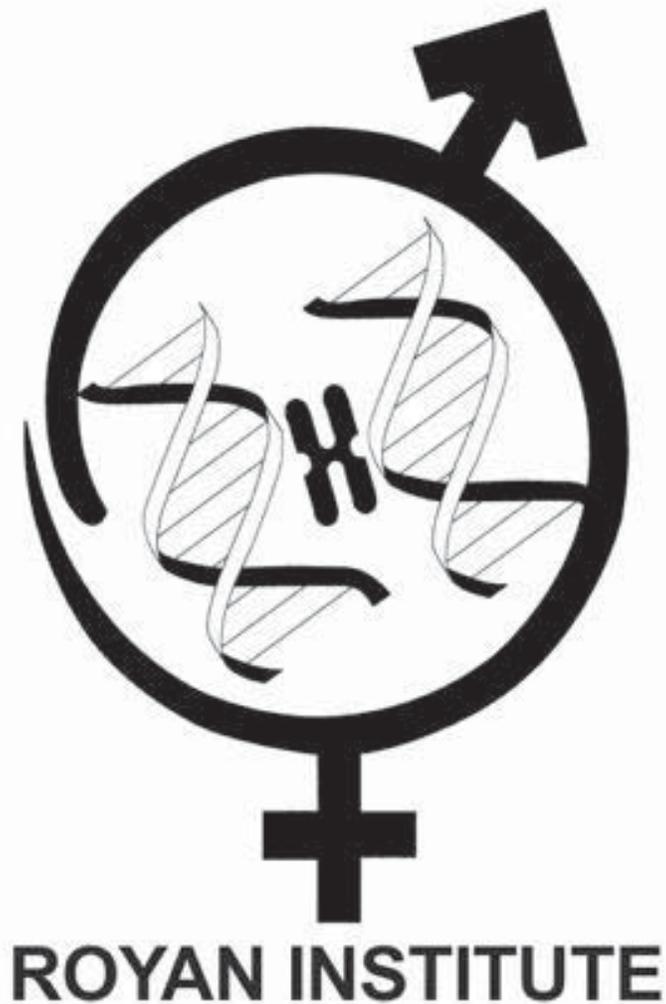


Abstracts of

**Royan International Twin Congress**  
**11<sup>th</sup> Congress on Reproductive Biomedicine**  
15-17 September 2010

**5<sup>th</sup> Royan Nursing and Midwifery Symposium**  
15-16 September 2010



Tehran, Islamic Republic of Iran

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**Abstracts of Royan International Twin Congress  
11<sup>th</sup> Congress on Reproductive Biomedicine**

**5<sup>th</sup> Royan Nursing and Midwifery Symposium**



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Pardadeh, Shahin  
Sabaghian, Marjan  
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Shakeri, Raheleh  
Zafarani, Fatemeh  
Zoghi, Fereshteh

## Contents

|                                   |     |    |
|-----------------------------------|-----|----|
| ● Scientific Board .....          | 1   |    |
| ● Collaborators .....             | 2   |    |
| ● Chairman Wellcome Message ..... | 3   |    |
| ● Invited Speakers                |     |    |
| ... Andrology .....               | 5   |    |
| ... Embryology .....              | 6   |    |
| ... Epidemiology and Ethics ..... | 12  |    |
| ... Genetics .....                | 23  |    |
| ... Reproductive Imaging .....    | 26  |    |
| ● Oral Presentations              |     |    |
| ... Andrology .....               | 29  |    |
| ... Embryology .....              | 33  |    |
| ... Epidemiology and Ethics ..... | 36  |    |
| ... Female Infertility .....      | 40  |    |
| ... Genetics .....                | 46  |    |
| ● Poster Presentations            |     |    |
| ... Andrology .....               | 52  |    |
| ... Embryology .....              | 60  |    |
| ... Epidemiology and Ethics ..... | 67  |    |
| ... Female Infertility .....      | 71  |    |
| ... Genetics .....                | 81  |    |
| ... Reproductive Imaging .....    | 82  |    |
| ● Nursing and Midwifery Symposium |     |    |
| ... Oral Presentation .....       | 84  |    |
| ... Poster Presentation .....     | 93  |    |
| ● Workshops and Courses .....     |     | 98 |
| ● Authors' Index .....            | 105 |    |

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Abbasi M.  
Abolhassani F.  
Abroun S.  
Adjaye J.  
Aflatoonian A.  
Aflatoonian R.  
Aghdami N.  
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Ahmadi SM.  
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Dickenson DL.  
Ding S.  
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Eftekhari Yazdi P.  
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Hartshorne G.  
Heimberg H.  
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Hoseini R.  
Hosseini A.  
Hosseini Salekdeh Gh.  
Hosseini SJ.  
Hosseinkhani H.  
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Jaenisch R.  
Jahdi F.  
Johansson L.  
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Kamrava M.  
Karimian L.  
Karimzadeh MA.  
Kazemeini SM.  
Khalaf Y.  
Khalili M.  
Korooji M.  
Kurpisz M.  
Laible G.  
Lee C. K. F.  
Mackay-Sim A.  
Madani T.  
Mahale S.  
Manik RS.  
Mc Elreavey K.  
Milliez J.  
Mohseni Meibodi A.  
Moini A.  
Moini M.  
Mojibian J.  
Mori T.  
Moser M.  
Movaghar B.  
Movahhedini M.  
Mukhopadhyay A.  
Nagy A.  
Nakatsuji N.  
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Nazari Tavakoli S.  
Neisani L.  
Nematollahi N.  
Niknejadi M.  
Norouzi MR.  
Pacey A.  
Parsa P.  
Parsanezhad E.  
Pebay A.  
Popov A.  
Poureisa M.  
Pourmand Gh.  
Ramezanzadeh F.  
Rashidi B.  
Redi CA.  
Rezazadeh Valojerdi M.  
Sabour D.  
Sadeghi MR.  
Sadighi Gilani MA.  
Sadrkhanlou R.  
Saeedi H.  
Salamonsen LA.  
Salehnia M.  
Salehpour S.  
Salman Yazdi R.  
Samani RO.  
Samsioe G.  
Saremi A.  
Sato E.  
Schlegel PN.  
Seyed Fatemi N.  
Shahhoseini M.  
Shahriari H.  
Shahverdi A.  
Shahzadeh Fazeli A.  
Sharma T.  
Shia M.  
Shiva M.  
Sills ES.  
Stamm C.  
Stojkovic M.  
Sugimura Y.  
Suzuki T.  
Svoboda P.  
Taheripanah R.  
Tahmaseb Pour A.  
Taylor H.  
Tehraninejad ESh.  
Ter Meulen R.  
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## Congress Chairman



**Nasser Aghdami**

**D**ear Friends and Colleagues;

On behalf of the organizing committee of Royan International Twin Congress, 11th Congress on Reproductive Biomedicine and 6th Congress on Stem Cell Biology & Technology, I would like to invite you to join us and attend this prominent event which will be held on September 15-17, 2010 in Tehran, Iran.

RITC-2010 will be held in conjunction with closing ceremony of the 11th Royan International Research Award, which contains five prizes for five best research projects in the field of Reproductive Biomedicine and Stem Cell Biology and Technology.

For the first time in 1999, when we had achieved the success of 1000 ART baby births, we decided to share this great experience with scientists and doctors from all around the world, and we held the first Royan International Research Award and Congress on Reproductive Biomedicine.

By September 2010, the time of 11th Royan International Congress, we will be reaching to the experience of more than 10,000 baby births resulting from the efforts of ART professionals and ART lab experts of the Royan Institute, and there would be too much to exchange with you throughout the scientific sessions of the congress.

Regenerative medicine and stem cells are of new hot scientific subjects which provide so many promising options for the future treatment of some major diseases such as cancers, nervous diseases, damaged organs and degenerative diseases.

In recent years the field of stem cell has witnessed enormous expansion in clinical and basic data. However the basic research is of vital interest to everybody working in this field and brings together scientists from all segments of basic and biomedical research and from various clinical disciplines, even if clinical applications to be developed finally require highly specialized knowledge and techniques.

Since 2006, we have been holding the Stem Cell Biology and Technology congress alongside the Reproductive Biomedicine part as a twin congress.

The scientific program of this twin congress will focus on breaking scientific developments and current issues from foremost opinion leaders. Each keynote speaker has been asked to not only review his/her particular area of expertise but also look ahead at possibilities for the next years.

The executive committee has also arranged social programs and pre/post congress tours to some historical cities of Iran like Isfahan, Shiraz, Kashan... for foreign guests to visit the cultural heritage and enormous tourism resources of our ancient country, Iran.

Hereby, I present my thanks to the participants of this conference, professors, young scientists and students! It is due to your keen interest that these fields are expanding and exploring new frontiers. We sincerely hope that each and every one of you has a wonderful time in Iran.

The organizers of Royan International Twin Congress are eagerly waiting to welcome you to this exciting extravaganza of knowledge and pleasure.

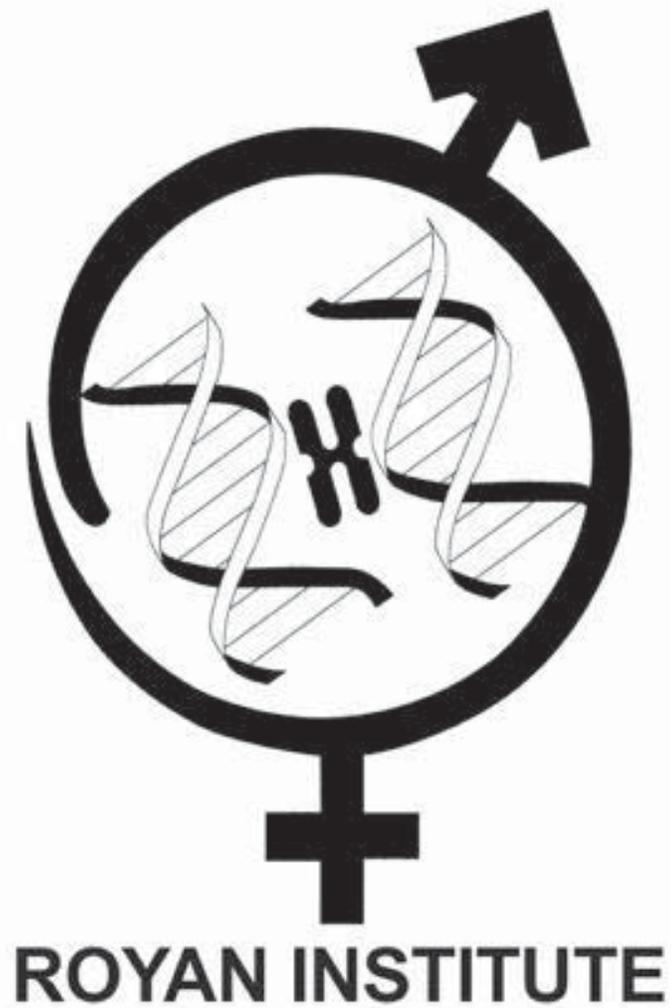
Best regards,

**Nasser Aghdami M.D./Ph.D.**  
**Congress Chairman**

Abstracts of

**11<sup>th</sup> Royan International Congress on Reproductive Biomedicine**

15-17 September 2010



Tehran, Islamic Republic of Iran

## Invited Speakers

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### Andrology

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#### I-1: Effect of High Intratesticular Estrogen on Spermatogenesis

Balasinor NH\*, D'Souza R, Upadhyay R

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**Background:** The presence of estrogen receptor beta and aromatase in the germ cell has highlighted the physiological role of the traditionally female hormone, estrogen, in spermatogenesis. Estrogen receptor alpha knockouts and aromatase knockouts have further accentuated the role of estrogen in germ cell maturation. To delineate effects of high intratesticular estradiol in the seminiferous epithelium and the mechanisms involved. The study was based on the fact that administration of exogenous estradiol suppresses the hypothalamus-pituitary gonadal axis (HPG) with a dose-dependant concomitant increase in intratesticular estrogen levels.

**Materials and Methods:** Three doses of 17- $\beta$  Estradiol, namely 20, 100 and 200  $\mu$ g/kg/day were administered subcutaneously to different batches of adult male rats for 10 days. The effect of the three doses on serum hormonal profile, intratesticular testosterone (T) and estradiol (E) levels and testicular morphology were studied. Further studies to delineate the mechanism causing spermiation failure was carried out with 100  $\mu$ g/kg/day for 10 days and the effect on Sertoli cell cytoskeleton and testis specific adherens junction was done by immunofluorescence and confocal imaging

**Results:** Twenty micrograms per kilograms per day of 17- $\beta$  estradiol affected the hypothalamus-pituitary axis, reducing serum gonadotropins and intratesticular testosterone; however, 100  $\mu$ g/kg/day of 17- $\beta$  estradiol decreased serum FSH and intratesticular testosterone, increased intratesticular estradiol, but had no effect on serum LH. Interestingly, 200  $\mu$ g/kg/day of 17- $\beta$  estradiol decreased serum and intratesticular T without any effect on serum gonadotropins. This could be attributed to the positive feedback effect of estrogens on gonadotropins. In the testis, morphologically two visible effects were seen, namely 'spermiation failure' in all three doses attributed to the suppression of T and FSH and a 'maintenance effect' in the 100  $\mu$ g/kg/day attributed to E and/or 10% of available intratesticular T. The direct effect of an increase in intratesticular estradiol levels was observed in terms of a decrease in apoptosis in germ cell. The study, therefore, suggests that 100  $\mu$ g/kg/day of 17- $\beta$  estradiol could be used to study the effects of high intratesticular estradiol with a concomitant decrease in intratesticular T and serum FSH levels on spermatogenesis. Hence further studies on mechanism causing spermiation failure were carried with this estradiol dose. Spermiation is the final phase of spermatogenesis lead-

ing to release of mature spermatids into the lumen of the seminiferous tubules. Morphologically, it involves a series of events, namely removal of excess spermatid cytoplasm, removal of ectoplasmic specialization, formation of tubulobulbar complex, and final disengagement of the spermatid from the Sertoli cell. Electron microscopic and confocal studies revealed an absence of tubulobulbar complex in step 19 spermatids after estradiol treatment, highlighting the significance of these structures in spermiation. It was further observed that treatment affected the Sertoli cell cytoskeleton and Arp2/3 complex that is critical for de novo polymerization of actin during tubulobulbar complex formation. In addition estradiol treatment also affected microtubule bundling and distribution of vimentin filaments in Stage VII-VIII of the seminiferous epithelium cycle suggesting an effect on Sertoli cell cytoskeleton.

**Conclusion:** The present study reports the role of 17- $\beta$  estradiol in inhibiting the formation of tubulobulbar complex, which could be one of the mechanism by which environmental estrogens influence male fertility.

#### I-2: Comparison of Transrectal Ultrasonography and Transrectal Ultrasonography-Guided Seminal Vesicle Aspiration in the Diagnosis of the Ejaculatory Duct Obstruction

Engin G<sup>1\*</sup>, Celtik M<sup>2</sup>, Sanli O<sup>2</sup>, Aytac O<sup>2</sup>, Muradov Z<sup>2</sup>, Kadioglu A<sup>2</sup>

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**Background:** To compare transrectal ultrasonography (TRUS) and TRUS-guided seminal vesicle aspiration in the diagnosis of ejaculatory duct obstruction (EDO). comparing the findings of TRUS and TRUS-guided seminal vesicle (SV) aspiration.

##### Materials and Methods:

**Design:** A retrospective case-controlled study comparing the findings of TRUS and TRUS-guided seminal vesicle (SV) aspiration.

**Setting:** Clinics of Urology and Radiology.

**Patient(s):** Seventy patients with suspected EDO (complete in 10, partial in 60 patients) on clinical evaluation.

**Intervention(s):** Each SV was punctured transrectally using a 20-gauge Chiba needle within 2 hours after ejaculation.

**Main Outcome Measure(s):** In SV aspirates, greater than three sperm per high-power microscopic field was considered a positive result for EDO.

**Results:** Fifty-five (78.6%) patients had evidence of EDO on diagnostic TRUS. However, obstruction on TRUS was confirmed in 49.1% (27 of 55) of the patients with SV aspiration. Higher sperm positivity rates were achieved in patients with SV dilation (11 of 13, 84.6%)

and prostatic midline/ED cyst (12 of 16, 75.0%). Stepwise logistic regression analysis revealed that the incidence of SV dilation was significantly higher, whereas that of chronic inflammatory findings in the prostate was significantly lower in the positive SV aspirate group.

**Conclusion:** TRUS alone is not a reliable tool for the diagnosis of EDO. For this reason, SV aspiration should be used as an adjunctive technique in patients with SV dilation or a prostatic midline/ED cyst to confirm the diagnosis before surgery.

**Keywords:** Male Infertility, Ejaculatory Duct Obstruction, Seminal Vesicles, Transrectal High-Intensity Focused Ultrasound, Guidance, Sperm Aspiration

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## Embryology

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### I-3: Fertility Preservation in Women with Cancer

Al-Hasani S

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As the efficacies of anticancer therapies were increase and quite efficient early diagnoses have been taken place in gynecological cancer, increased long-term survival of cancer patients and long-term complications of anticancer treatments are being encountered. The lost of ovarian reserve or function due to gonadal toxicity is the major problem that mainly focused.

A wide range of new fertility preservation options and/or techniques, although the majority of them are experimental, are now available prior to oncological treatments. Ovarian transposition, a surgical preservation technique, is one of these methods that avoid the gonadal tissue from radiation field in order to prevent the gonadal toxicity of radiotherapy. Partial and/or total (experimental) extraction, subsequent cryopreservation and re-transplantation of ovarian tissue, another surgical preventive option both for radiotherapy and chemotherapy side-effects, is expected to be a paramount method in the future that was performed in selective cases previously. Particularly, medical methods with such agents like GnRH analog or antagonists, and Danazole have been reported to prevent chemo or radiotherapy related gonadal toxicity. Cryopreservation of oocytes, zygotes and embryos either by vitrification or slow-rate freezing subsequent to ovarian stimulation and oocyte pick-up along with/without traditional IVF and intracytoplasmic sperm injection are other common preventive techniques.

Currently cryopreservations of the oocyte, zygote and/or embryos are seems to be the most effective methods in fertility preservation especially in reproductive age women. In regard of the cryopreservation tech-

niques vitrification has claimed to be most appropriate technique due to simplicity and cost-effectiveness. On contrary, surgical methods are still accepted as difficult, and costly alternatives, as well experimental ones such as re-transplantation are not reported to be significantly efficient and reliable alternatives. In parallel, the effects of medical preventive alternatives, those generally resulted in pseudo-menopausal situation and mainly recommended to patients prior to reproductive age, are still controversial concerning side-effects, reduced effectiveness.

The number of available techniques for conserving fertility has increased in the last decade, but large studies are still needed to draw a conclusion. Some of these effective options such as vitrification of embryos are not allowed in Germany. Other technical issues remain unresolved, as is the question of medical insurance reimbursement for the most efficient procedures. All current aspects and techniques should be compared in order to presume the best method and standardization in female fertility preservation. New approaches and current management on fertility preservation in gynecological cancers were discussed in this presentation.

### I-4: Is It Time Now to Cancel Fresh Embryo Transfer

Al-Hasani S

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The implantation of embryo depends on the quality of the embryo and the receptivity of the endometrium. While the factors affecting embryo quality were optimized, the implantation rates did not reach the desirable levels. This directs the blaming for endometrial receptivity which already has been affected by supra-physiological levels of hormone, namely estradiol, in controlled ovarian hyperstimulation (COH) cycle. On the other hand, cryopreservation, especially the vitrification method, has been highly improved to give almost the same results of fresh state, in addition to decreased rate of blastogenesis birth defects. All these encourage us to put our opinion to overcome the problem of endometrial receptivity by cancellation the fresh embryo transfer (fresh ET) and to perform the frozen-thawed embryo transfer (FET) in cases of excessive response to COH.

### I-5: Molecular and Cellular Interactions in Uterine Receptivity for Implantation

Aplin JD

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**Background:** Though plausible candidate adhesion systems have been identified, current knowledge of embryo-maternal attachment in human is limited by the inability to conduct well-controlled functional investigations. We have sought a viable medium-throughput model for the identification and functional assessment of molecular markers in the initial epithelial phases of implantation. An ideal model should bypass the scarcity of human embryos and well known difficulties in obtaining, establishing and maintaining confluent, normal, well-differentiated endometrial epithelium *in vitro*.

**Materials and Methods:** Blastocysts flushed from day 4 pregnant superovulated mice were transferred to confluent human Ishikawa cell monolayers. Cells were untreated or steroid primed (E2 followed by MPA and E2) and characterised using a panel of differentiation markers. Embryo attachment was tracked by phase contrast microscopy, and weakly and stably attached embryos identified. Apically displayed cell surface glycoproteins were biotinylated using periodate/hydrazide, recovered by avidin affinity and analysed using a proteomics protocol.

**Results:** After 48h of co-culture, 85% of blastocysts had attached loosely, but only 40% attached stably to the epithelial cell surface. In contrast, 95% of embryos attached stably to tissue culture plastic. This demonstrates that weak attachment of a majority of embryos is followed by stronger adhesion of a smaller proportion. Initial attachment is efficient either in the presence or absence of hormone, but steroid priming (E2 followed by MPA and E2) increased stable attachment from 40 to 70%. Initially, stable attachment occurred without disruption to the integrity of the epithelial monolayer, but clearance of surface MUC1 occurred beneath and adjacent to attachment sites. Later, lateral spreading of embryonic cells was accompanied by displacement of subjacent epithelial cells.

Biotinylation was demonstrated to be highly vectorial, with label confined to the apical epithelial surface. The proteomic protocol led to the identification of approximately 30 species, about half of which are already recognised as endometrial cell surface glycoproteins. We tested endometrial tissue by immunofluorescence to confirm the presence of novel markers in luminal epithelium of midsecretory phase endometrium. Surprisingly, adhesion molecules present predominantly in the lateral membrane domain are also detectable in smaller amounts at the apical surface. siRNA knock-down reduces expression, but this leads to impairment of lateral cell adhesion and epithelial monolayer integrity, with implications for epithelial penetration by the implanting embryo.

**Conclusion:** The model shows sufficient resemblance to key features of human and mouse implantation to be useful as a first line of discovery for cellular and molecular hypothesis building. Based on the observations, implantation *in vivo* may arrest when embryos fail to progress from initial to stable attachment. Novel candidate adhesion systems of possible importance for em-

bryo-maternal interaction *in vivo* are being tested.

## I-6: Remodelling Uterine Spiral Arteries in Pregnancy

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**Background:** During the first trimester of pregnancy the uterine spiral arteries that supply blood to the placenta are remodelled, creating heavily dilated conduits lacking maternal vasomotor control. To effect permanent vasodilatation, the internal elastic lamina and medial elastic fibres must be degraded. Failure of remodelling is a key characteristic of the pathological placenta and is thought to be a primary causative mechanism in pre-eclampsia and fetal growth restriction. We sought to identify the elastolytic proteases involved and their source. Maternal (vascular smooth muscle cells, uterine NK cells and macrophages) and fetal (extravillous trophoblast) cells are all potentially involved in ECM remodelling.

**Materials and Methods:** We examined cells *in situ* in vessel walls at various stages of transformation, and used a combination of *in vitro* methods to specify and test molecular mechanisms.

**Results:** Primary first trimester cytotrophoblasts (CTB) derived from the placenta exhibited intracellular and membrane-associated elastase activity; membrane-associated activity was primarily attributable to matrix metalloproteinases (MMP). Affymetrix microarray analysis and immunocytochemistry implicated MMP-12 (macrophage metalloelastase) as a key mediator of elastolysis. Cultured human aortic smooth muscle cells (HASMC) exhibited constitutive membrane-associated elastase activity and inducible intracellular elastase activity, and also expressed MMP-12 protein. A specific inhibitor of MMP-12 significantly reduced CTB- and HASMC-mediated elastolysis *in vitro*. MMP-12 is expressed by interstitial and endovascular trophoblast in the first trimester placental bed and by vascular SMC (VSMC) in remodelling spiral arteries. Perfusion of isolated spiral artery segments with CTB-conditioned medium stimulated MMP-12 expression in medial VSMC.

**Conclusion:** These data support a model in which trophoblast and VSMC utilize MMP-12 cooperatively to degrade elastin during vascular remodelling in pregnancy, with the localized release of elastin peptides and CTB-derived factors amplifying elastin catabolism. Other evidence indicates that maternal uNK cells and macrophages also contribute to elastin degradation and vessel wall disruption.

## I-7: Maternal Signalling to the Placenta

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**Background:** Though it is well established that maternal blood-borne signals influence highly the growth of the placenta, the mechanisms are not known. *In vitro* trophoblast culture models are limited by an inability to reconstruct the polarised bilayer of the human hemochorial placenta. We have used a first trimester villous tissue explant system to investigate how growth factors interact with placental cells. We have in particular been interested in how signals arriving at the microvillous membrane of the post-mitotic syncytium can stimulate proliferation in cytotrophoblast progenitor cells.

**Materials and Methods:** Tissues were obtained at pregnancy termination (8-13 weeks). Explants were established and maintained in serum-free DMEM/F12 and siRNA knockdown achieved either in the syncytium alone or in cytotrophoblast as well. Avidin-conjugated quantum dots were loaded with biotinylated growth factor for tracking by high resolution microscopy. Explants were incubated with BrdU before fixation and immunostaining to detect cells that had passed through S phase during culture. IGFs or TGF $\beta$  were used to stimulate placental tissue. Phosphorylation pathways were detected immunochemically.

**Results:** IGF-I and -II stimulate proliferation in cytotrophoblasts through a pathway that depends on IGFR1. Downstream activation of the activating tyrosine phosphatase SHP-2 occurs in cytotrophoblast but not in the syncytium, leading to MAP kinase-dependent proliferation and PI3 kinase-dependent protection from apoptosis. IGF-conjugated quantum dots were taken up by syncytiotrophoblast and delivered to the underlying cytotrophoblast. ERK phosphorylation occurs in syncytium in response to IGF, indicating the existence of an independent signalling pathway that might be targeted directly at syncytial function. TGF $\beta$ 1 stimulates cytotrophoblast proliferation via T $\beta$ RI/II and not T $\beta$ RV. SMAD2 is activated in both cytotrophoblast and some syncytiotrophoblast nuclei, again suggesting that multiple downstream pathways are targeted as a result of ligand binding to the syncytial surface. SMAD2 is also activated in response to another ligand, IGFBP3, but no pERK is seen and proliferation is inhibited in both the presence and absence of IGF.

**Conclusion:** Syncytiotrophoblast plays a selective as well as a protective role, decoding and transmitting specific signals to the underlying cytotrophoblast in order to maintain or expand the progenitor cell layer. When considering treatments for fetal growth restriction, targeting signalling components downstream of receptor may be needed to discriminate between growth and differentiation effects.

### **I-8: Somatic Cell Nuclear Reprogramming by Mouse Oocytes Endures Beyond Reproductive Decline**

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**Background:** The mammalian oocyte has the unique feature of supporting fertilization and normal development while being able of reprogramming the nuclei of somatic cells towards pluripotency, and occasionally even totipotency. Whilst oocyte quality is known to decay with somatic ageing, it is not a given that different biological functions decay concurrently. In this study, we tested whether oocyte's reprogramming ability decreases with somatic ageing, as previously reported for natural reproduction.

**Materials and Methods:** We collected oocytes from B6C3F1 mice aged beyond the usual reproductive age (climacteric; 57-62 week-old) and from B6C3F1 mice in young age (6-8 week-old). We randomly allocated the oocytes of each age group to two subgroups: one subgroup to be analyzed for gene expression by the Agilent microarray, and the other subgroup to be used for somatic cell nuclear transfer (SCNT) from cumulus cells. Resultant cloned embryos were cultured to morula and blastocyst stage and analyzed for developmental rates, cell physiology and gene expression.

**Results:** We show that oocytes isolated from climacteric mice yield ooplasts that retain reprogramming capacity after SCNT, at levels similar to ooplasts of young donors. Despite differences in transcriptome between ooplasts of old and young mice, gene expression profiles of the resultant SCNT blastocysts were very similar.

**Conclusion:** Our observations strongly suggest that the outcome of oocyte-induced reprogramming is determined by 1) the dependence on the availability of intrinsic reprogramming factors tightly regulated throughout ageing, as well as 2) intracellular and environmental checkpoints during pre-implantation development, towards selection of the successfully reprogrammed embryos. While oocytes are not regenerated but rather last for life, we further propose that these cells can still be a resource for somatic reprogramming when they cease to be considered safe for sexual reproduction. Last, we note that while transcription factor-induced reprogramming becomes less efficient with somatic ageing, oocyte-induced reprogramming does not, hinting at intrinsic differences between these reprogramming methods.

### **I-9: Important Components in Human Embryo Culture Media**

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### **I-10: Quality Control in IVF Lab**

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## I-11: Optimizing the ART Outcome

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

In my talk I will try to cover both the staff and the patient's perspectives of an optimal treatment – from construction, equipment, disposables, Total Quality Management (TQM) and treatment procedures.

The location for the clinic should be selected carefully, since high traffic areas and polluted air has a negative outcome of the treatment. As a proactive preventive measure the clinics HVAC system is fitted with HEPA and charcoal filters (Me, VOCs) and that building materials and furniture that do not contain or release toxic compounds into the laboratory environment.

A logistic layout of the clinic and location of equipments reduces the time spent to perform the procedures and is thereby more efficient, thus saving money for the clinic. All essential equipments are plugged into sockets connected to an uninterrupted electrical power supply (UPS), which eliminates effects of power failure on ongoing treatments. Incubators are supplied with an uninterrupted inflow of medical grade CO<sub>2</sub>. After proper installation the equipments are calibrated with certified testing equipments and their performance continuously followed and documented.

After selection and installation of equipment and purchasing of embryo-tested disposables (evidence-based medicine) the premises are thoroughly cleaned and evaluated. In order to obtain and maintain an optimal level of patient care and success rates, a Total Quality Management (TQM) system (covers all aspects of the clinic and the procedures, from facility parameters, initial evaluation of a patient, stimulation protocols, culture and embryo transfer techniques, gestational period, to parameters in connection with the delivery of the baby) must be implemented. Implementation of a TQM system is associated with a huge workload and requires investments. However, due to increased standardization and efficiency of all processes and procedures, improved transparency and traceability of all actions performed, the quality of service improves substantially, which is of benefit for both the customer and the staff. TQM is not only designed to detect and eliminate problems, but also to constantly improve the clinic's performance by incorporate latest know-how and techniques, as well as to make all procedures and processes more effective and reproducible.

It is essential that all staff members knows exactly how, why and when everything is to be performed, which standardizes the routines and optimizes the outcome of the patient's treatment cycle. Standardization is obtained by the introduction of standard operational procedures (SOPs) that can be of technical (equipments) or clinical type. The SOPs are upgraded yearly and the latest international technologies and bench mark processes and procedures are incorporated in a never-ending project. Indicators are an important tool for auditing of the clinic's performance over time, for detection of gradually declining results, performance of individual staff members and areas of need for education. The indicators should be internationally defined and recognised, which reduces misinterpretations and facilitates internal as well as international communication and auditing. Unfortunately, many clinics only focus on the ultimate outcome – pregnancy rates, which do not assess the overall quality of the ART program.

The laboratory is run by an experienced embryologist, who interacts with representatives for the clinicians and nursing, for the creation of an optimal treatment tailored to the expectations and medical background of the couple.

The management of staff is highly neglected and it is therefore very important to develop a staff friendly environment, where highly trained, motivated and skilled staff members with high self-esteem develops, thrives and are retained. Do not underestimate the effect that a staff-friendly environment has on the outcome of the treatments.

Despite that TQM of an ART clinic is a never-ending effort, costly, time-consuming and a lengthy process, the many positive outcomes that this leads to is highly beneficial for the clinic, staff and especially the patients. There is no doubt that a standardization of the many processes within the clinic facilitates the communication between staff, patients and other clinics. Remember that there are no short-cuts to perform a work of excellence and that all staff categories must be involved and committed.

A couple that undertakes a treatment in a "controlled" environment will be given an optimal and safe treatment in a safe environment by highly educated, skilled and professional staff members. One should also remember that the procedures are performed within facilities especially designed for ART, equipped with calibrated certified ART-equipments, using embryo-tested disposables, media products and procedures, proven to give high reproducible and consistent pregnancy rates. The couples are therefore given high value for money, but it also simultaneously reduces the time spent for auditing and increases the inflow of patients to the clinic.

## I-12: Physiological ICSI

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Prior to ICSI the cumulus-oophorous-complex of the oocyte is commonly removed with impure animal-derived hyaluronidase of low strength. The lower strength of the enzyme, the high concentration of impurities and the necessity of extensive time-dependent mechanical denudation damages the oocytes, increases the risk of lysing the oocytes and the transfer of animal-derived pathogens. All this can be avoided by using a highly pure, non-toxic and effective recombinant human hyaluronidase, which requires less mechanical denudation, reduces oocyte damage, increases fertilization, embryo quality and pregnancy rates. During the ICSI procedure the sperm are subjectively selected. However, it is now possible to Backgroundly select motile sperm, via their hyaluron receptors, of a better morphology, higher chromatin stability and lower incidence of aneuploidy, which improves fertilization rates, embryo quality, blastulation and ongoing pregnancy rates - "Physiological ICSI".

**Keywords:** Hyaluronidase, ICSI Cumulase, Hyaluronan binding sites, HBA, SpermSlow, PICSI, DNA, Embryo

### I-13: Complete Fertilization Failure after ICSI: What to Do?

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Fertilization *in vivo* is a complex process consisting of six steps, namely, penetration of the cumulus complex, binding of the sperm to the zona pellucida, fusion of oocyte and sperm, oocyte activation, sperm remodeling, and formation of the pronuclei. Since ICSI bypasses the first three steps any problem related to fertilization failure after ICSI arises from oocyte activation onwards. According to literature approximately 10% of all patients suffering from severe male factor infertility and 5% with insuspicious sperm count could be affected by this dilemma. Currently three methods are available to treat such patients: usage of an ionophore, a modified ICSI according to Tesarik, or accumulation of highly active mitochondria on the site of fertilization.

### I-14: Group Culture in Human Is Superior to Individual Culture in Terms of Blastulation, Implantation and Life Birth

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**Background:** In contrast to animal data, group culture does not increase preimplantation development in humans. This prospective study was done to test a new type of culture dish allowing for individual culture and any possible effect of autotrophic factors.

**Materials and Methods:** Within a 6-month period, 72

patients with  $\geq 9$  fertilized eggs were enrolled in this prospective evaluation. Their 936 zygotes were split into three subgroups (individual culture, individual culture with contact to neighbours, group culture). All concepts were cultured in 30 $\mu$ l drops (medium change on day 3) until blastocyst stage. On day 5, a single blastocyst transfer was performed and the remaining blastocysts of good quality vitrified.

**Results:** Fertilization rates were 69% for IVF and 81% in ICSI. Blastulation was 48%. Single blastocyst transfer resulted in a clinical pregnancy rate of 54%. Group culture was superior in terms of compaction ( $p < 0.01$ ) and blastulation ( $p < 0.001$ ) as compared to individual culture. A better blastocyst quality was observed in group culture ( $p < 0.05$ ). As a trend, more live births were achieved when blastocysts derived from group culture were transferred ( $p = 0.07$ ).

**Conclusion:** This is first evidence that in human grouping embryos improves preimplantation development. Based on these results it is recommended that culture volume should be reduced or embryo density increased.

### I-15: Survival and Development Competence of Buffalo Preantral Follicles Using Three Dimensional Collagen Gel Culture System

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**Background:** The aim of the present study was to develop a three-dimensional (3D) collagen gel culture system for the *in vitro* growth and survival of buffalo preantral follicles with or without growth factors.

**Materials and Methods:** Buffalo ovaries were collected from a local abattoir and preantral follicles were isolated through microdissection. Isolated preantral follicles were put either in collagen gel coated culture dish or embedded in a microdrop of collagen gel. The culture medium was TCM-199 fortified with fetal calf serum (10%), insulin transferin selenium solution (ITS, 1%), epidermal growth factor (EGF, 20 ng/ml) and follicle stimulating hormone (FSH, 0.5 $\mu$ g/ml). Follicles were divided into three groups and cultured in the medium described above (group a, control), with addition of insulin like growth factor (IGF-I, 100 ng/ml, group b), or with addition of IGF-I and basic fibroblast growth factor (bFGF, 10 ng/ml, group c). Preantral follicles were incubated at 38.5 °C in 5% CO<sub>2</sub> and maximum humidity. Culture medium was replenished after every 72 h and spent medium was stored at -30°C for hormone analysis.

**Results:** We found that the extracellular matrix of collagen gel maintained follicle viability and growth by providing surface interaction and increasing attachment of follicles. Preantral follicles embedded in collagen gel droplets had better antrum formation and development

as compared to the whole surface coated culture method. Follicles cultured with IGF-I on collagen gel matrix showed a significantly ( $p < 0.05$ ) higher survival rate and larger mean diameter of follicles on day 10 of culture with improved growth and mucification as compared to the control group. However, follicles cultured in the combination of IGF-I with bFGF had decreased survival rate and smaller mean follicles diameter than the IGF-I group (b). Progesterone (P4) accumulation was greater on day 9 of culture in follicles cultured in IGF-I as compared to control; whereas, P4 was markedly decreased in the combination of IGF-I with bFGF. Follicles of the control group could survive for up to 10–15 days before degenerating, but follicles cultured with growth factors were able to survive up to 20 days and showed signs of early antrum formation.

**Conclusion:** In summary, we have shown that collagen gel was a novel and efficacious 3D microenvironment for the extended culture of buffalo preantral follicles. Supplementation of culture medium with growth factors was found to be essential for antrum formation.

**Keywords:** Buffalo, Preantral Follicle, Collagen, IGF-I, bFGF

### **I-16: Computer Aided Sperm Analysis and Sperm Functional Testing (Hyperactivation) as Background Tools in the Evaluation of Sperm Function/Quality**

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After twenty years Computer Aided Sperm Analysis has developed considerably and is now routinely used in many laboratories across the world. The advantage of the CASA methodology available is twofold: Analysis of particularly sperm concentration, sperm motility, sperm morphology and vitality is quantified in an Background manner. Secondly, most of the CASA analysis with the exception of sperm morphology, is rapid and literally takes a few seconds. These two factors allow scientists to produce data in an Background and consistent manner and also make experiments possible that would otherwise have had too many time constraints. In this paper emphasis will be placed on quantitative sperm motility and its multiple uses in assessing sperm function in general fertility studies; testing the effect of a vast range of substances (peptides, hormones, toxicology) on sperm function.

Two approaches will be described. In each instance either sperm washing, swim-up or differential centrifugation were used. When sperm were placed in media supporting sperm function and largely simulate the composition of follicular fluid after washing, a certain percentage of sperm would become hyperactive. In good quality sperm usually more than 20% of sperm become hyperactive

when CASA parameters are set at  $VCL > 150$ ;  $LIN < 45$  and  $ALH > 7$ . Unfortunately sperm from different individuals exhibit maximum hyperactivation at different time intervals over three hours. However, in cases where a specific strategy is required for a particular patient, additional tests such as "the hyperactivation test" may be useful. Also, the ability of a sperm sample to become hyperactivated can be used to assess the effect of substances such as pesticides and hormones on functional integrity. A second useful CASA functional test that has been developed in our laboratory, and used in certain hormone assays is the percentage rapid moving sperm in a sample and a cut-off point of  $VCL > 100$  to 120 was found most useful when washed samples were employed.

In conclusion, CASA now provides us with tools which are reliable and consistent to study sperm function as related to fertility and toxicology in a quantitative and Background way.

### **I-17: The Importance of Animal Sperm Models in Understanding Human Male Fertility/Infertility**

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Male fertility assessment is performed in the context of WHO guidelines. Currently the 5<sup>th</sup> edition of the WHO guidelines have been published and technicians and clinicians are confronted with a new set of criteria. Apart from coming to grips with these criteria it is accepted/assumed that human fertility is declining? However, both aspects are debatable. The accuracy/consistency of semen evaluation according to WHO can only be considered as a course measure for the potential of male fertility. Secondly, evidence that male fertility is declining globally is debatable since most semen analysis represents examples of patients that visit fertility clinics. Furthermore, more infertility clinics and better access to these clinics would in principle provide a biased semen sample.

It may then be argued which alternatives are available to study the problem of male fertility? The hypothesis developed in this study is that human semen quality should be viewed in the context of the evolution of sperm competition. Which animal models provide a model for studying human infertility?

Two models have been selected for this study. Firstly, the naked rodent mole which is the only eusocial mammalian species. This model is one which represents major stress in the colony and most males are rendered sub-fertile except for the breeding male. Secondly, animals as diverse as dolphin and elephant provide extreme cases of sperm competition and in the case of the dolphin also promiscuity. In bottlenose dolphin there ap-

pears to be extreme sperm competition with about 95% normal sperm and above 90% motility.

Naked rodent moles experience a considerable amount of stress and this is very well reflected in their very poor/ semen/sperm characteristics. Of particular interest is that they also have a very low percentage abnormal sperm and low percentage motility (like in humans). In contrast, dolphins have >90% normal sperm morphology and >80% motility. Furthermore, in elephant and rhinoceros semen parameters in general are also excellent when field conditions are good. It is proposed that human sperm characteristics be viewed in an evolutionary context. The low level of promiscuity in humans may have selected against the basic principles of sperm competition and accordingly sperm characteristics are poor. It is proposed that the "general poor quality of human sperm" does not necessarily represent poor quality sperm but is a consequence of evolutionary selection. Many different animal models assist to understand sperm quality in a phylogenetic context.

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## Epidemiology and Ethics

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### I-18: The German law in Assisted Reproduction

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### I-19: From 'Designer Babies' to 'Transhumans': the Ethics of Enhancement Technologies

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**Background:** To evaluate the medical evidence and ethical reasoning behind the claims of enhancement technologies such as gene transfer and neurocognitive stimulation.

**Materials and Methods:** Analytical survey of the literature on enhancement technologies in terms of their philosophical presuppositions and evidence base, including the World Health Organisation Report Genomics and World Health (2002) and the European Commission Science and Technology Options Assessment (STOA) (2009), together with a critique of the findings of the European Commission Enhance project.

**Conclusion:** The enhancement technologies have occasioned strong and conflicting reactions, from Julian Savulescu's assertion that we ought to be putting neurocognitive enhancers into the public water supply,

to George Annas's denunciation of germline genetic transfer as 'genetic genocide.' We need to avoid premature optimism about the success of such technologies, almost all of which have yet to undergo first-in-human clinical trials, and to be careful in distinguishing the more speculative ventures from established technologies like preimplantation genetic diagnosis (sometimes sensationistically labelled 'creating designer babies'). Caution in interpreting the evidence base needs to be matched by philosophical scepticism about the claim that we can benefit the common good by pursuing enhancement technologies at the expense of less glamorous technologies. Although most ethicists who favour enhancement technologies are philosophical utilitarians, even on a utilitarian basis we can save more lives and do more global good by purifying water and sending mosquito nets to malarial zones. Of newer biotechnologies, those most likely to improve Third World health include affordable simple diagnostic tools developed through modified molecular genomics, and recombinant technologies to develop effective vaccines against common diseases. These measures, rather than enhancement technologies, should be the priority of anyone who wants to use biotechnology for the common good.

The enhancement technologies, however, are typical of 'me medicine': all about the individual's private, personal benefit. They promote the notion that I have a right or even a duty to be the best 'me' possible, to transcend my human limitations. By contrast, mosquito nets and water purification, as well as the more complex genomic technologies mentioned above, are examples of 'we medicine'. Over the past two hundred years it is 'we medicine' that has produced the greatest reduction in mortality and morbidity.

Enhancement technologies also risk broadening the chasm between the genetic 'haves' and 'have-nots'. 'Transhumanism' or 'posthumanism' is the extreme case of enhancement, using biotechnology to slip the bonds of being human. In a posthuman world, the 'naturals' would become the 'disabled,' leaving the disabled to an even more uncertain fate.

As the WHO report on *Genomics and World Health* states, genetic enhancement is 'a dereliction of the real duty of health care, which is 'helping to secure equality of opportunity for persons whom serious disease and disability undermine. Genetic enhancements of normal function, on the other hand, do not serve justice in this way.' An uncritical advocacy of enhancement technologies, I similarly conclude, is neither good medicine nor good ethics.

### I-20: Good Science and Good Ethics@ Why We Should Discourage Payment for Eggs in Stem Cell Research?

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**Background:** To evaluate current scientific and legal trends in provision of human eggs for stem cell research and to propose a policy which is both ethically and scientifically sound

**Materials and Methods:** Literature survey of European and US policies on payment for egg donation in somatic cell nuclear transfer (SCNT) research; comparative analysis of potential for success in SCNT and induced pluripotent stem cell (iPSC) research

**Results:** While the New York State Stem Cell Research Foundation has recently voted to allow payment for eggs in SCNT research, their decision is at odds with legal trends in other jurisdictions, with the aftermath of the Hwang case, with National Institute of Health guidelines, and with scientific trends favouring the development of iPSC research over SCNT.

**Conclusion:** Although past ethical controversies pitted 'good science' against 'good ethics'--the former supposedly requiring human eggs for stem cell research, the latter arguing that this was ethically problematic because it exploited egg donors--good science and good ethics are no longer contradictory. We should discourage payment for human eggs for research because it leads neither to good science nor to good ethics.

**Keywords:** Stem Cell Research, Oocytes, Research Ethics

### **I-21: Embryo Relinquishment for Reproduction**

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**Background:** The conceptualization of the transfer of embryos between the individuals who created them to one or more recipients for family-building is hotly contested – particularly as regards whether the practice should be most appropriately considered to be “donation” or “adoption”. This paper examines this debate, considering the research carried out on the intentions and decisions of those with embryos to relinquish and ethical debate over how this process should be organised.

**Materials and Methods:** Examination of the medical, sociology and ethics literature.

**Conclusion:** Given the diversity of views on embryo relinquishment reflected in the studies carried out in this area, those with unused embryos should have the choice over how they relinquish their embryos. A 'one size fits all' model of embryo relinquishment, based on a medical model of disinterested donation could be restrictive, 'Options need to be explored that respect individuals' reproductive goals and values.' As long as those options do not harm others, the principle of reproductive choice can be used to justify couples having options available to them that they find both morally and practically suitable.

### **I-22: Ethical Issues in Everyday Infertility Practice**

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**Background:** Infertility is a speciality that has attracted considerable attention, focussing mainly on 'controversial' issues (such as saviour siblings) and national policy. Whereas, relatively little is known about how infertility clinicians approach ethical decisions in their everyday practice. This study aims to develop a deeper understanding of this by examining how infertility clinicians construct the ethical aspects of their practice.

**Materials and Methods:** Twenty-two qualitative semi-structured interviews were conducted with infertility clinicians in the UK. Interviews lasted on average an hour and were tape recorded and transcribed. A modified grounded theory method of data collection and analysis was used.

**Results:** The study found that the process by which ethical decisions were made was of key importance to the clinicians: such decisions were seen as most appropriately taken by a group reaching a consensus, to ensure impartiality and consistency. The study also found a number of key issues that the clinicians found ethical troubling the main ones being: whether the donor should be anonymous; how to apply welfare of the child criteria when assessing recipients for treatment; and treating same-sex couples. These and other issues raised during the study will be discussed and explored. The implications of these findings for how ethical decision-making can be strengthened both in infertility units and general clinical practice will be discussed.

### **I-23: Freezing Oocytes: New or Old Ethical Issues?**

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**Background:** This paper considers whether the development of oocyte freezing raises any new or distinctive ethical issues. It will examine the ethical issues raised by this technique: the 'social' use of egg freezing and how that might be defined; the use of egg freezing for cancer patients; and limitations and benefits of the technique.

**Materials and Methods:** A consideration of the literature and an ethical analysis of the issues raised by the developments in egg freezing.

**Conclusion:** This paper concludes by arguing that egg freezing does not, in itself, raise any new ethical issues,

but casts existing debates over safety of treatments, appropriate age of parents, who should be a parent, and possible increase in technological interventions in reproduction in a new light.

## **I-24: Consents and Contracts for Embryo Cryopreservation**

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**Background:** Couples receiving IVF treatment may choose to have embryos frozen, with the aim of creating a pregnancy and ultimately a live birth in future. Problems can arise when couples separate. Under UK law, embryos can only be transferred with the consent of both genetic parents. The issue came to public prominence in the case of Natallie Evans, who lost the opportunity to have her own genetic children after her relationship ended and her partner withdrew consent for embryo storage. Additional issues arise where one of the genetic parents is a gamete donor, or has died or become incapacitated. This study examines the current consent laws in the UK, and explains the possible case for changes that would allow couples a choice of consent agreements.

**Materials and Methods:** A possible change in the law, to give couples increased flexibility in their consent agreements, is considered. One type of agreement would be as at present, allowing either genetic parent to withdraw consent at any time before embryo transfer. The other type of agreement would involve one member of the couple signing away the right to withdraw consent, so that their partner would have control over the embryo. The couple would then decide between themselves which type of consent agreement to sign. The benefits of and possible objections to this change in the law are analysed according to philosophical and legal principles.

**Results:** The proposed change in the law may directly benefit both members of a couple in a situation where they would not create frozen embryos together under the current law but would do so under the proposed new law. For example, a woman in the position of losing her fertility to urgent medical treatment may be unwilling to have embryos created with her partner and frozen if the man retains the right to withdraw consent at a later date. It is then in the man's interests to be able to sign away the right to later withdraw consent. More generally, the change gives couples more autonomy by allowing them a choice, and does not remove any option currently open to them. It might be particularly suitable for gamete donors, because withdrawal of consent by a gamete donor, though rare, has serious impact upon recipients.

Possible objections to change take three main forms: (i) concern about the welfare of a child born to parents who have separated; (ii) concern about any broader costs to

society from the birth of such children; and (iii) concern that a genetic parent who signed away his or her right to withdraw consent would be the victim of an unfair contract. It is shown that the first objection would imply that a baby born to parents not together has a life not worth living; this seems absurd and also conflicts with the general principle under English law that 'wrongful life' is not recognised. Both (i) and (ii) conflict with the legality and practice of offering fertility treatment to single women in the UK, and the removal of the 'need for a father' in the UK Human Fertilisation and Embryology Act 2008, instead requiring 'supportive parenting'. For (iii), it is generally accepted that a necessary condition for a contract to be unfair is that the wronged person is either likely to be in an unfit state of mind or has insufficient information at the time of signing the contract. Thorough counselling, with mandatory individual counselling for each partner, may answer possible concerns about unfair contracts.

Some practical considerations remain, such as the time periods over which embryos can be used under different consent agreements.

**Conclusion:** There is a strong case for revising the law to allow a choice of consent agreements when patients opt to have embryos frozen. Robust consent-advisory procedures will be needed, helping couples to think through their consent decisions more thoroughly than at present.

## **I-25: Gamete Cryopreservation for Cancer Patients, Ethical Considerations**

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Cancer patients often receive gonadotoxic treatments, such as radiation or certain chemotherapies. The doses and regimens used for treatment vary in different individuals and cancers. Recent progress has improved the survival rates of patients with cancer, often using highly aggressive therapies and combinations. This results in growing numbers of cancer survivors, some of whom will be sterile or have compromised fertility.

For men, storage of sperm before cancer treatment starts should be routine. Sperm storage is safe and effective. The illness, or an underlying deficit, can result in suboptimal sperm quality, however, modern fertility treatments, such as ICSI, can overcome most sperm deficits. Sperm freezing is not feasible for azoospermic men, or pre-pubertal boys. Methods of storing stem cell stages with potential to develop into sperm are not yet available clinically.

For women, oocyte storage is more difficult. Few oocytes are available, ovarian stimulation is required which might exacerbate estrogen-receptor positive breast cancer,

ovarian stimulation is timed according to the menstrual cycle which could delay starting cancer treatment, and the pregnancy rates using frozen oocytes are low in many centres (but increasing with vitrification).

An alternative for women is ovarian cortex freezing. This is storage of immature, primordial follicles in pieces of the peripheral ovarian tissue, removed by surgery. The tissue can be thawed and transplanted back, allowing the follicles to grow *in vivo* when the patient wants a pregnancy. In future, growth of follicles might be achieved *in vitro* to perform IVF, eliminating risks of reintroducing cancer cells in the transplanted tissue. Only ~20 pregnancies have arisen from ovarian cortex freezing, so this is still a research procedure.

#### **Ethical considerations:**

Many issues are raised by both the storage of gametes, and their application in cancer patients.

1. Who should be offered fertility preservation? Should there be age limits or other eligibility criteria? Currently this decision relies upon the cancer clinician who may or may not refer the patient for fertility preservation.
2. To what extent should preparations for life after cancer affect the treatment itself? Are delays acceptable for fertility preservation if they may affect the patient's survival chances? Who should take this decision?
3. Storage of gametes separates them from the person. Decisions about the stored gametes in the event of incapacity or death need to be made in advance. Accidents and incidents can result in loss of stored material. For how long should gametes be kept?
4. These decisions affect others who have an interest in the patient's fertility, such as partners, parents and family members. Should these people be able to influence the decisions or the use of stored gametes? For example, should partners have access to the gametes if the cancer patient dies? Should children be born from gametes of the deceased?
5. Is the welfare of the potential child affected? When the patients want to use the stored material, are they really cured? 5 year survival is a widely used survival index for cancer patients, but for parenthood a longer period of survival is desirable. How important is life expectancy when any of us may die at any time?

This presentation focuses on ethical considerations of fertility storage and presents original data on the views of cancer clinicians about sperm storage.

#### **I-26: Considerations for PGD Applications in Elective Human Embryo Sex Selection**

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The promise of medical innovation has long evoked social commentary, particularly when personal reproduc-

tive autonomy may be impacted. Development of the oral contraceptive, effective and safe surgical sterilization, and later IVF and ICSI are among the revolutionary developments where initial reaction was dubious, but eventually, each was accorded mainstream status as clinical experience accumulated. Debate about the moral and social implications of these treatments accompanied their introduction into the medical marketplace. This pattern appears to be repeating itself as preimplantation genetic diagnosis (PGD) is used specifically for elective sex selection of human embryos. As with prior challenges in reproductive medicine, developing meaningful "guidelines" for this latest controversy has proven to be a contentious task. Indeed, the progression of ethics committee reports from the American Society for Reproductive Medicine seems to echo the ambivalence within society at large regarding this issue. Sex selection claims have been based on sperm sorting, while flow cytometry and especially PGD have facilitated this selection at the gamete and embryo stage, respectively. Yet, patient demand, market forces and practitioner considerations associated with the application of PGD for this have not received much formal study. Increased physician familiarity with PGD is a welcome trend, and clinicians should prepare for important questions from patients about the risks and benefits of this technology. While the advances of PGD are relatively new, the desire for sex selection is not. Those who contemplate offering PGD for this purpose should first clarify their own personal moral position, and then evaluate each clinical circumstance on a case-by-case basis. This approach is consistent with the policy that IVF patients should have full access to PGD for elective sex selection, but that physicians should first use "moral suasion" to promote offspring sex by chance, even when modern reproductive technologies could be applied to influence the outcome. While some clinicians and policy makers may find the use of PGD for elective embryo sex selection problematic, regulatory measures mandating its elimination would be even more worrisome and objectionable. Since a resolution satisfactory to all interested parties is unlikely to be achieved, continued multidisciplinary study as proposed by professional organisations seems appropriate.

#### **I-27: Determining the Status of Non-Transferred Embryos in Ireland: A Conspectus of Case Law and Implications for Clinical IVF Practice**

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The development of *in vitro* fertilisation (IVF) as a treatment for human infertility was among the most controversial medical achievements of the modern era. In Ireland, the fate and status of supranumary (non-transferred) embryos derived from IVF brings challenges both for clinical practice and public health policy because there is no judicial or legislative framework in place to address the medical, scientific, or ethical uncertainties. Complex legal issues exist regarding informed consent and ownership of embryos, particularly the use of non-transferred embryos if a couple separates or divorces. But since case law is only beginning to emerge from outside Ireland and because legislation on IVF and human embryo status is entirely absent here, this matter is poised to raise contractual, constitutional and property law issues at the highest level. Our analysis examines this medico-legal challenge in an Irish context, and summarises key decisions on this issue rendered from other jurisdictions. The contractual issues raised by the *Roche* case regarding informed consent and the implications the initial judgment may have for future disputes over embryos are also discussed. Our research also considers a putative Constitutional 'right to procreate' and the implications EU law may have for an Irish case concerning the fate of frozen embryos. Since current Medical Council guidelines are insufficient to ensure appropriate regulation of the advanced reproductive technologies in Ireland, the report of the Commission on Assisted Human Reproduction is most likely to influence embryo custody disputes. Public policy requires the establishment and implementation of a more comprehensive legislative framework within which assisted reproductive medical services are offered.

### I-28: Gamete Donation from An Islamic View

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One of the distinction between an ethical proposition and a proposition in Islamic Jurisprudence (Feqh) or Islamic Law is in their predicates. All three speak of the free action of human being as their subjects.

Nature of human being discussed in this article according to Islamic point of view. Status of a gamete and nature of human being correlated to each other and affect to the conclusion. Human being has two separate level of existence: first is before creation of a spiritual living and second, after this moment. this may affect our discussion about gamete donation.

We can change our destiny by choices we made during our life. While at the previous ages before flourishing these technologies, we attribute our inabilities to God's will, now we can attribute our abilities to our permitted choices. This view accords with Islamic teachings.

Nevertheless, we encounter difficulties we made it by these choices and now an active and live religion should

show new ways of life style. This article tries to consider religious requirements of an Islamic style of the life regarding these new choices.

This Article also attempts to consider Islamic values, presupposing Fatwas of Ulama. First, investigate normative values of gamete donation and then have a penetrate look to what is accorded with Islamic scriptures - if any. These two sections discussed independently and then we consider the effects of the second section on the first as an Islamic Ethics.

**Keywords:** Islamic Ethics, Gamete Donation, Islamic Values, Halal, Haram

## Female Infertility

### I-29: Can Fresh Embryo Transfers Be Replaced by Cryopreserved-Thawed Embryo Transfers in Assisted Reproductive Cycles? A Randomized Controlled Trial

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**Background:** Controlled ovarian stimulation (COS) has been shown to advance endometrial maturation and affect adversely implantation in assisted reproduction technology (ART) cycles. It has been reported that there is a better embryo-endometrium synchrony in frozen-thawed embryo transfer (FET) cycles than fresh embryo transfer (ET) cycles. The Background of this study was to compare ongoing pregnancy rate between fresh ET and FET cycles in ART. According to recent published prospective study from Belgium and our research, the pregnancy outcome after frozen embryo transfer is comparable or even better than pregnancy outcome after fresh embryo transfer. Some studies showed that Vitrification may increase the embryo survival rate and the chance of pregnancy rate.

**Materials and Methods:** In a prospective, controlled study, the patients who were classified as high responders, were randomized to either fresh ET or FET. The embryos in FET group were cryopreserved with vitrification by Cryotop method. Randomization was done on the day of ET according to a computer-generated random numbers. Ongoing pregnancy rate was the primary outcome measure.

**Results:** A total of 374 patients were included, 187 of which were randomized to FET and 187 to fresh ET. There were 39% (n= 73) ongoing pregnancy in FET group compared with 27.8% (n= 52) in fresh ET group [odds ratio (OR) = 1.66; 95% confidence interval (CI) = 1.07-2.56; p<0.05]. Implantation, clinical pregnancy and multiple pregnancy rates were also higher in FET group.

**Conclusion:** FETs can be performed instead of fresh ETs to improve the outcome of ART cycles in highly se-

lected patients.

**Keywords:** Fresh Embryo Transfer, Frozen-Thawed Embryo Transfer, Vitrification, Endometrial Receptivity, Ongoing Pregnancy

### **I-30: Separate and Combination Effect of Sex Hormones on TLRs Expression in Fallopian Tubes**

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**Background:** Implantation is characterized by the interaction of two immunologically and genetically distinct tissues. The embryo differs from the cells of the mother, and would be rejected as a parasite by the immune system of the mother if it didn't secrete immunosuppressive agents. Thus, immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss. Most of the recent investigations suggest differences in the expression of some immune factors in women with recurrent miscarriage.

Toll Like Receptors (TLRs) are the main family of pattern recognition receptors and they recognise pathogen-associated molecular pattern and constitute a major part of the innate immune system. Reports from our laboratory and others have demonstrated the existence of TLRs in the female reproductive tract and also it's known that they expressed highly during secretory phase of menstrual cycle in endometrium which is the time of implantation. However, little has been done to identify if TLR expression in the fallopian tubes is cycle dependent as well. In addition, it is not known if sex hormones can influence TLRs expression in fallopian tubes. The aims of this study were to test the existence of TLR1-6 genes in fallopian tube and also, combination and separate effects of sex hormones on the expression of these receptors in an immortalised human fallopian tube epithelial cell line (OE-E6/E7).

**Materials and Methods:** RT-PCR was used to show the existence of TLR1-6 genes in fallopian tube tissue and OE-E6/E7 cell line. To compare relative quantities of TLR 1-6 genes expression in OE-E6/E7 cell line, they were treated by different levels of estradiol and progesterone separately, they were divided into ten groups; control (without any additional treatment of sex hormone), E0.1 (0.1nM/ml estradiol), E1 (1nM/ml estradiol), E10 (10nM/ml estradiol), E100 (100nM/ml estradiol), P1

(1nM/ml progesterone), P10(10nM/ml progesterone), P100 (100nM/ml progesterone) and P1000 (1000nM/ml progesterone) respectively. In addition, The OE-E6/E7 cell line was treated by both estradiol and progesterone in combination and they were divided into four groups; control (without any additional treatment of sex hormone), Menstruation (1nM progesterone and 0.1nM estradiol), Pre-ovulation (6.5nM progesterone and 1.5nM estradiol) and window of implantation (35nM progesterone and 1nM estradiol). Relative TLRs 1-6 expression quantities were compared between these groups using real time quantitative PCR.

**Results:** TLR1-6 genes were expressed in human fallopian tube tissue and OE-E6/E7 cell line. Our data clearly showed that Estrogen had no effect on the expression of TLRs in OE-E6/E7 cells. In contrast, progesterone had an inhibitory effect on the expression of TLR1-4 genes in this cell line. However, the expression of TLRs 1-6 was altered in OE-E6/E7 with different concentrations of sex hormones in combination. The highest expression of all the TLR genes was in window of implantation group, compared to all other groups.

**Conclusion:** It seems sex hormones alter the expression of some of the TLRs in human fallopian tube epithelial cells *in vitro*. Although increasing levels of sex hormones in combination enhanced TLR1-6 genes expression in OE-E6/E7 cells, further experiments are in progress to elucidate the regulatory mechanism behind this novel effect of sex hormones in modulating innate immunity in the human female reproductive tract. Therefore, Understanding the roles of local and systemic immune factors in fallopian tube and uterine for implantation is necessary to develop approaches to enhance reproductive health and fertility in humans.

**Keywords:** Innate Immunity, Fallopian Tube, Toll Like Receptor, Estrogen, Progesterone

### **I-31: ART Outcome in Endometrioma**

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**Background:** Our Background was to evaluate the effect of ovarian endometrioma on ovarian stimulation outcomes in Intra Cytoplasmic Sperm Injection (ICSI).

**Materials and Methods:** In this prospective cohort study, we followed 103 patients underwent ICSI procedures at royan Institute during 10 month. The study group consisted of 46 women who had endometrioma

with size more than 1 cm. The control group included 57 patients with male-factor infertility. The standard long protocol with gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle stimulating hormone (rFSH) was used for ovarian stimulation. Two groups were compared for number of oocytes retrieved, oocytes grades, embryo quantity and quality. We also performed inter group comparison in patients with unilateral endometrioma.

**Results:** There were no significant differences about basal characteristics between endometrioma and control groups. Our results also showed similar endometrial thickness, follicle numbers, and good embryo grades (grade A or B) in compared groups. Two groups had similar pregnancy rate.

However, patients with endometrioma had higher gonadotropin consumption compared with control group. The mean number of retrieved oocytes in patients with endometrioma was  $6.6 \pm 3.74$  compared with control group  $10.4 \pm 5.25$  ( $p < 0.001$ ). The numbers of metaphase II oocytes were also significantly lower in patients with endometrioma ( $5 \pm 3.21$  vs  $8.2 \pm 5.4$ ).

In patients with unilateral endometrioma, we could not find any significant differences about main outcome measures between normal ovary and involved ovary with endometrioma.

**Conclusion:** It seems that ovarian endometrioma affects ovarian response in stimulation phase by decreasing the numbers of retrieved oocytes but not affecting quantity and quality of embryos or pregnancy rate.

**Keywords:** ICSI, Ovarian Endometrioma, Number of Oocytes, Embryo Quality, Cycle Outcome

### I-32: Implantation and Recurrent Pregnancy Loss

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**Background:** Recurrent pregnancy loss (RPL), defined as 3 or more consecutive pregnancy failures, is a common and distressing disorder. Chromosome instability in the conceptus is the most common cause whereas uterine factors are invariably invoked to explain non-chromosomal miscarriages. These uterine factors are, however, poorly defined.

**Materials and Methods:** Laboratory-based analysis of endometrial biopsies, primary endometrial cultures and co-cultures with human blastocysts.

**Results:** Implantation coincides with the differentiation of endometrial stromal cells (ESCs) into specialized decidual cells, which in pregnancy control trophoblast invasion and placenta formation. We investigated the soluble factors involved in crosstalk between decidualizing ESCs and the implanting embryo using a human co-culture

model. Multiplex immunoassay analysis of culture supernatants demonstrated that decidual cells selectively recognize developmentally impaired embryos and respond by inhibiting the secretion of a panel of implantation and inflammatory modulators. Undifferentiated cells do not mount such a response, suggesting that adequate decidualization of the endometrium is essential not only for normal pregnancy but also for recognition and elimination of compromised conceptions. We demonstrate that the ability of ESCs to express a decidual phenotype is grossly impaired in RPL patients and characterized by prolonged and enhanced expression of the pro-implantation cytokine prokineticin-1 (PROK1) and attenuated prolactin (PRL) production, a sensitive marker of the decidual response. RPL was further associated with a complete dysregulation of PROK1 and PRL expression in response to human chorionic gonadotropin signaling. We postulated that impaired maternal recognition and selection of invasive but compromised embryos would lead to enhanced fecundity, defined by short time-to-pregnancy (TTP) intervals. To test this hypothesis, we analysed the TTP in 2076 pregnancies reported by 560 RPL patients. Woman-based analysis of the mean and mode TTP showed that many RPL patients are highly fecund and 40% of patients can be considered 'super-fertile', defined by a mean TTP of 3 months or less.

**Conclusion:** Decidualization of the endometrium is essential to establish a functional feto-maternal interface. We found that decidual cells also selectively recognize compromised human embryos and, conversely, that failure to express an adequate decidual phenotype disrupts the maternal response to embryonic signals and negates embryo selection at the time of implantation. These observations indicate that the decidualizing endometrium serves as a biosensor for embryo quality and establish a unifying mechanism for both euploidic and aneuploidic recurrent pregnancy failure.

### I-33: Oxidative Stress Responses in Early Pregnancy

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**Background:** Survival of the conceptus is dependent on continuous progesterone signaling in the maternal decidua but how this is achieved under conditions of oxidative stress that characterize early pregnancy is unknown.

**Materials and Methods:** Laboratory-based analysis of endometrial biopsies and primary endometrial cultures.

**Results:** Using primary cultures, we show that modest levels of reactive oxygen species (ROS) increase sumoylation in human endometrial stromal cells (HESCs), leading to enhanced modification and transcriptional in-

hibition of the progesterone receptor (PR). The ability of ROS to induce a sustained hypersumoylation response, or interfere with PR activity, was lost upon differentiation of HESCs into decidual cells. Hypersumoylation in response to modest levels of ROS requires activation of the JNK pathway. Although ROS-dependent JNK signaling is disabled upon decidualization, the cells continue to mount a transcriptional response, albeit distinct from that observed in undifferentiated HESCs. We further show that attenuated JNK signaling in decidual cells is a direct consequence of altered expression of key pathway modulators, including induction of MAP kinase phosphatase 1 (MKP1). Overexpression of MKP1 dampens JNK signaling, prevents hypersumoylation, and maintains PR activity in undifferentiated HESCs exposed to ROS.

**Conclusion:** JNK silencing uncouples ROS signaling from the SUMO conjugation pathway and maintains progesterone responses and cellular homeostasis in decidual cells under oxidative stress conditions imposed by pregnancy.

### **I-34: Steroid Hormone Signalling at the Feto-maternal Interface**

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**Background:** Progesterone is indispensable for differentiation of human endometrial stromal cells (HESCs) into decidual cells, a process that critically controls embryo implantation. However, HESCs also abundantly express androgen receptors (AR), yet the role of this member of the superfamily of ligand-dependent transcription factors in the decidual process remains poorly elucidated.

**Materials and Methods:** Laboratory-based analysis of endometrial biopsies and primary endometrial cultures.

**Results:** Although HESCs express both PR and AR, activation of the cAMP pathway is critical to sensitize the cells to progesterone as well as androgens. We demonstrate that cAMP signaling attenuates ligand-dependent sumoylation of both PR and AR, which enables these receptors to become strong transcriptional activators. In fact, decidualization is associated with global hypo-sumoylation and redistribution of SUMO-1 conjugates into distinct nuclear foci. This altered pattern of global sumoylation is not attributable to impaired maturation of SUMO-1 precursor or altered expression of E1 (SAE1/SEA2) or E2 (Ubc9) enzymes but coincided with profound changes in the expression of E3 ligases and SUMO-specific proteases. Down-regulation of members of the protein inhibitors of activated STAT (PIAS) family upon decidualization pointed towards a role of these E3 ligases in PR and AR sumoylation. We demonstrate that PIAS1 serves as the E3 SUMO ligase for both PR and AR and that loss of PIAS1 in decidualizing cells is es-

sential for steroid hormone signalling

By combining small interfering RNA technology with genome-wide expression profiling, we also found that AR and PR regulate the expression of distinct decidual gene networks. Ingenuity pathway analysis implicated a preponderance of AR-induced genes in cytoskeletal organization and cell motility whereas analysis of AR-repressed genes suggested involvement in cell cycle regulation. Functionally, AR depletion prevented differentiation-dependent stress fibre formation and promoted motility and proliferation of decidualizing cells. In comparison, PR depletion perturbed the expression of many more genes, underscoring the importance of this nuclear receptor in diverse cellular functions. However, several PR-dependent genes encode for signaling intermediates and knockdown of PR, but not AR, compromised activation of WNT/ $\beta$ -catenin, TGF $\beta$ /SMAD and STAT pathways in decidualizing cells.

**Conclusion:** These findings demonstrate how dynamic changes in the SUMO cycle mediated by the cAMP pathway determine the endometrial responses to progesterone and androgens. We further show that the activated AR and PR exert non-redundant functions during the decidualization process. Whereas the role of AR is centred on cytoskeletal organization and cell cycle regulation, PR regulates HESC differentiation, at least in part, by re-programming growth factor and cytokine signal transduction.

### **I-35: Europe: Declining Fertility Despite IVF**

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Although fertility rates are falling in many countries, Europe is the continent with the lowest. As men and women choose to have fewer children, total fertility rates in Europe have fallen below replacement levels: for a total fertility rate of 1.5, the rate is around 1.7 children per woman for the cohorts born in 1965. This is due to individual decisions arising from the instability of modern partnerships and the high cost of maintaining a family. These decisions are easy to implement today, with the widespread use of contraception and abortion to reduce the incidence of unplanned pregnancy.

Government policies have only limited effect on fertility rates, whether they involve cash to families for pregnancy and child support or payments for assisted human reproduction. In contrast, efforts to improve education can achieve better quality of life and higher economic growth at the societal level. Support for education and compatibility of work and family life are probably the strategies most likely to improve prosperity in the long term and enable couples to have the family size they want.

This review assesses trends in fertility rates, explores

health and social factors, and looks at the impact of health and social interventions designed to boost fertility rates.

### **I-36: Influence of Lifestyle Factors on Spontaneous and Induced Fecundity**

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Several features of today's lifestyles, particularly the increasing numbers of women remaining childless and the general tendency to postpone childbearing, explain the low fertility in many countries. A substantial proportion of postponers are not fully aware of the steep drop in fecundity after the age of 30-35; other women know about the loss but place their confidence in the "magic" powers of IVF; many others know about the decrease but nevertheless postpone childbearing because of a lack of acceptable alternatives. In fact, IVF cannot make up for all births lost by postponement and although vitrification seems to hold out promise for indefinitely storing oocytes for social reasons it is still an experimental procedure.

Other lifestyle variables can contribute to reducing fecundity at any age, and the following in particular merit examination:

- undernutrition in the woman
- overweight in women and men
- smoking and alcohol use by either sex.

Obesity in particular - a very frequent problem - raises the risk of infertility and reduces the success of all pro-fertility treatments. Smoking too (30% of women in the rich countries) can reduce fecundability and cause premature reduction of the ovarian reserve. In addition, smoking during pregnancy causes a loss of ovarian follicles in the offspring.

### **I-37: Medical Treatment of Endometriosis in Infertility**

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There is still not enough evidence that endometriosis per se and infertility are causally related. While extensive pelvic adhesions or the loss of ovarian tissue clearly bears out the causative role of endometriosis this is more difficult in other patients. Several randomized controlled trials and observational studies have in fact reported almost uniform negative results:

- spontaneous pregnancy rates were comparable in patients with unexplained infertility with and without pelvic endometriosis;

- medical and surgical "suppressive" treatments in patients with pelvic endometriosis did not convincingly change their fecundity;

- donor insemination and IVF cycles in women with and without endometriosis did not produce different outcomes.

Only a small donor insemination study seems to show an impairment of the quality of the oocytes produced by women with endometriosis. Even patients with retrovaginal implants have apparently normal fertility.

Endometriotic ovarian cysts are found in 20-40% of women with endometriosis and may greatly alter the ovarian cortex structure leading to a significant decline of spontaneous and induced ovulation. The most severe defects of ovarian function are seen in cases with bilateral endometriosis. Surgical treatment of the cysts is also associated with a decline in spontaneous and induced ovulation. Frequently endometrioma recurs after ablation but long-term use of oral contraceptives offers specific protection against this risk.

### **I-38: Effect of Ovarian Stimulation on Endometrial Receptivity**

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### **I-39: Identification of Oviductal Protein(s) that Modulate Pre-Implantation Embryo Development**

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### **I-40: Implantation Failure**

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**Background:** The process of implantation involves the interaction of the human embryo and the uterine epithelium. Failure of implantation is a major reason for infertility in women and the inability to achieve endometrial receptivity is responsible for much of the failure of reproductive technologies. Management of repeated implantation failure despite transfer of good-quality embryos still remains a dilemma for ART specialists. The aim of this review is to evaluate the different aspects of implantation failure investigation and management.

**Materials and Methods:** Pubmed, Medline, Cochrane

research

**Results:** Several factors, including embryo quality, and cellular and molecular changes in endometrium may contribute to the insufficient fetomaternal interaction resulting in reproductive failure. Genetic or metabolic abnormalities of the embryo are very important in preventing of implantation and embryonic growth. The efficacy of preimplantation genetic screening (PGS) in repeated IVF failure is questionable but the studies suggest that young patients with RIF may not benefit from PGS. Local and systemic immune factors, cytokines, and growth factors may interact with adhesion molecules. So, heparin and aspirin can overcome the antibodies such as APA, AEA (anti endometrial antibodies) effects and other matrix-associated proteins, glycoproteins, and peptides. Although, leukemia inhibitory factor (LIF) is an important factor in the human implantation process but the recombinant LIF was not associated with higher pregnancy rate. Recently, it was found that the injury-derived inflammation in the biopsy-treated patients provokes the immune system to generate an inflammatory reaction and generates a focus for uterine dendritic cells accumulation that, in turn, enhances the endometrial expression of essential molecules, which facilitate the interaction between the embryo and the uterine epithelium.

**Conclusion:** A better understanding of the mechanisms underlying endometrial receptivity and implantation should guide clinicians through proper management and treatment of infertility and implantation failure, and may eventually enable widespread adherence to single embryo transfer practices.

**Keywords:** Implantation Failure, PGD, Immunologic Factors, Endometrial Receptivity

#### **I-41: Remove of Hydrosalpinx Prior the ART Cycle**

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#### **I-42: The Role of Blastocyst Transfer and PGD in Improving of Result in Patients with Recurrent Implantation Failure**

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#### **I-43: Investigation and Treatment of Couples with Recurrent Miscarriage in Royan**

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Recurrent abortion is classically defined as three or more consecutive pregnancy losses, although recently more than two pregnancy losses also considered as recurrent abortions. This condition affects approximately 1% of couples at reproductive age.

Recurrent pregnancy loss has been attributed to anatomic uterine pathologies, genetic defects, endocrine disorders, immunologic factors, prothrombotic state and environmental factors, but 40-50% of cases remain classified as having unknown etiology.

The risk of recurrence increases with the maternal age and number of successive losses. Women with previous pregnancy losses have a higher abortion risk (25%) than women with previous successful pregnancy (5%). Based on the data of recently published large randomized controlled trials and meta analyses the recommendation of ESHRE, special interest group for early pregnancy loss should include obstetric and family history, age, BMI and exposure to toxins, full blood count, antiphospholipid antibodies (Lupus anticoagulant and anticardiolipin antibodies), parental karyotype, pelvic ultrasound or hysterosalpingogram. We will present the risk factors of recurrent abortion in patients Referred to Royan Institute.

#### **I-44: Long-Term Sequelae of Polycystic Ovary Syndrome: Gynecological Cancer**

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Women with polycystic ovary syndrome (PCOS) have been reported to be at increased risk of a number of gynecological neoplasias, including endometrial, breast, and ovarian cancer. The data supporting an increased risk is almost entirely inferential, based primarily on small case series or shared risk factors.

One of the difficulties in exploring the association between these cancers and PCOS, is that they remain primarily diseases of postmenopausal women and present long after PCOS has faded. Only a fraction of cancer cases present in pre-menopausal women, where a concurrent diagnosis of PCOS may exist.

The basis for the concerns about long-term sequelae is that women with PCOS have a number of reproductive and metabolic abnormalities. The reproductive abnormalities include chronic anovulation, prolonged exposure to estrogen, progesterone deficiency, and androgen excess, which may contribute to an increased risk for gynecological cancers in which the hormonal milieu is an important contributor to etiology and prognosis.

Endometrial cancer is currently thought to be perhaps the best example of a hormone-dependent neoplasia. Endometrial cancer is thought to arise from prolonged exposure to estrogen, without the benefits of progesterone,

a condition known as unopposed estrogen. Estrogen is a clear mitogen on the endometrium and leads to proliferation of both the glandular and stromal components.

More recently, insulin resistance and hyperinsulinemia have been implicated as contributory agents to a variety of neoplasias. Smaller studies of women with endometrial cancer have shown increased fasting and glucose-stimulated insulin levels compared with controls. Another study found a similar result among women with endometrial cancers both in relation to controls and women with other hormone-dependent neoplasias such as breast cancer. A variety of mechanisms has been proposed.

Insulin is a powerful mitogenic influence on a variety of tissues including endometrium and breast epithelium, and this proliferative effect may contribute to the appearance of oncogenes and transformation of benign tissue. *In vitro* studies of cancer cell lines have shown that insulin is mitogenic, and most cell cultures of tumor cells require the presence of insulin in order to survive.

Insulin may play a role in the development of estrogen receptor negative endometrial cancers- cancers that usually are more aggressive and have a poorer prognosis.

A link between PCOS and cancer of the breast appears probable on both theoretical grounds, although the epidemiological evidence is mixed. An association between PCOS and ovarian cancer seems unlikely, leaving aside the putative association with ovulation-inducing drugs, which is an issue that should not apply to the modern approach to unifollicular ovulation induction in patients with PCOS and anovulatory infertility.

#### **I-45: Endometriosis and Infertility: Surgical Approaches to Treatment**

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The quantity of Infertility in woman reproductive age suffering from endometriosis in Russia is from 15% (V. Baskakov) to 38% (Popov A). But the treatment success not exceeding 45-48% (not randomized). And the correlation between prevalence endometriosis rate and PR is absent.

Is There a Role For Hormonal Treatment in Endometriosis-Associated Infertility? Suppression of ovarian function to improve fertility in minimal-mild endometriosis is not effective and should not be offered for this indication alone. There is no evidence of its effectiveness in more severe disease.

Does Surgery for Minimal-Mild Disease Improve Pregnancy Rates? Ablation of endometriotic lesions plus adhesiolysis to improve is effective compared with diagnostic laparoscopy alone. Does Surgery for Moderate-Severe Disease Improve Pregnancy Rates? The fertility in minimal-mild endometriosis role of surgery in improv-

ing pregnancy rates for moderate-severe disease is uncertain. The effect of surgery for peritoneal disease is small. Excision of rectovaginal lesions is of doubtful value and associated with severe morbidity. First-line surgery for the large ovarian endometriomas seems to be the procedure with the most favorable balance between benefits, harm and costs (<http://www.guideline.gov>). A practical advantage of surgery is temporary pain relief in symptomatic patients. This may render feasible spontaneous attempts at conception in women who refuse or prefer to postpone IVF (Vercellini P). There are the pitfalls of surgical treatment of endometriosis; there are non-adequate surgery for diagnosis and staging and non-adequate first operative procedure. We shouldn't use high energy to remove the endometriomas, bipolar coagulation (max 30 Watt) and/or suture are more safety.

Is *In Vitro* Fertilisation (IVF) Indicated? IVF is appropriate treatment, especially if tubal function is compromised, if there is also male factor infertility, and/or other treatments have failed. Treatment with a GnRH agonist for 3 to 6 months before IVF in women with endometriosis increases the rate of clinical pregnancy. Diphereline 3,75 mg in Russian clinical practice – effective alternative daily forms medications (immediate release). But IVF PR (pregnancy rate) are lower in women with endometriosis than in those with tubal infertility (I-II st. - 16,6%, III-IV st. - 14,9%, tubal Infertility - 37,4%). According our date (not RCT) SL (super long) Protocol of IVF in case of deep endometriosis increased PR (14% vs 25%).

#### **Conclusion:**

- The effective of surgery for endometriosis-associated infertility may be overvalued.
- The second surgery for infertility treatment is not effective. It should be better to do IVF.
- Complete and detailed information on risk and benefits of treatment alternatives must be offered to patients, in order to allow unbiased choices between different possible options (Vercellini P. et al, 2009)

#### **I-46: Reproductive Surgery in IVF Époque**

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IVF was one of the best discoveries in the XX century. Till 2006 more than 4 000 000 children were born after IVF (Mouzon J. 2009).

Today we should find the right balance between reproductive surgery and IVF in order to increase the effectiveness of pregnancy rate (PR). It is very important for the treatment of endometriosis-associated and peritoneal factor of sterility.

However the success of surgical treatment of sterility in endometriosis has been overestimated. The actual PR

after laparoscopic treatment is not more than 25% and less than 15% in the cases of severe endometriosis. We can increase the PR on 45% if we apply IVF timely.

We find very important to safe ovarian reserve when the endometrioma of ovary is removed as much as possible (preoperative estimation of ovarian function, application of safe energy, urgent IVF etc.)

Laparoscopic treatment is only effective in I-II degree of adhesions. But it is more advisable to perform IVF in the cases of III-IV degree of adhesions.

We recommend to remove sactosalpinx before IVF which will decrease the risk of tubal pregnancy and pyosalpinx, as well increase PR on 45 to 53% (Popov et al. 2007).

Is it necessary to remove asymptomatic myomas before IVF systematically? There are no convincing data that sub serous and intramural myomas influence fertility unfavorably (Klatsky et al. 2008). However myomectomy in cases of sub mucosal myoma and myoma fast growth are compulsory before pregnancy.

It is important there is no singular case if rapture of uterus in 360 pregnancies after myomectomy by laparoscopic and hysteroscopic approaches.

The patients should be produced the comprehensive and detailed information about the risks and advantages of alternative treatments to provide Background and unbiased option among different kinds of sterility treatments.

#### **I-47: ART Outcome in PCOS Patients**

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PCOS is still a problem in gynecology and infertility. Especially in ART the problem of PCOS patients is bigger and more highlighted. These patients are prone to over and under stimulation. Strategies to overcome hyper stimulation are: use of metformin, ovarian drilling, use of minimal stimulation, suppressing LH with use of OCP or GnRh analogues, use of GnRh antagonist in COH.

But none of these strategies are completely accepted and there are still questions about their efficacy. To overcome the problem of under stimulation, there are more difficulties. Solution like step up gonadotropin protocols, use of GnRh antagonist instead of agonist, use of minimal stimulation is still under debate. Other problem in ART of PCOS patients is the high rate of empty follicle syndrome, the higher proportion of eggs in Germinal vesicle or metaphase stage. In the detail of lecture we unill go through all these.

#### **I-48: Hysteroscopic Surgery**

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## **Genetics**

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### **I-49: Human Y Chromosome Proteome Project**

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The success of the Human Genome Project (HGP) has provided a blueprint for the approximately 20,000 gene-encoded proteins potentially active in all of the hundreds of cell types that make up the human body. Yet we still have limited knowledge about a majority of the gene-encoded proteins which are the “building blocks of life” and “cellular machinery”. It is estimated that for nearly half of the proteins encoded in the human genome, there is no experimental evidence for their protein existence and for many others there is very little information related to protein abundance, localization, and function. Therefore, Human Proteome Organization (HUPO) propose a gene-centric approach to generate a human proteome map with an “information backbone” about the proteins expressed from each gene locus, and to make this information publicly available with no restrictions, as was done with the genome sequence data. Thereby, a gene-centric human proteome project will facilitate in-depth studies to understand human biology and disease. In Iran, the current efforts are focused on mapping the proteome of human chromosome Y, a project endorsed by HUPO. The Y chromosome is unique under many aspects. It is always in the haploid state and full of repeated sequences but it is responsible for important biological roles such as sex determination and male fertility. The goal of human Y chromosome Proteome Project (YHPP) is to fill the void between genotype and phenotype for basic science discovery and clinical application. Reasonable end-points of YHPP must be clearly defined, feasible within a limited time-period and achievable without paradigm shifts in technology. We propose a systematic approach to ensure that, for each predicted protein-coding gene, at least one of its major representative proteins will be characterized in the context of its major anatomical sites of expression, its abundance and its functional relevance in a biological and/or medical context. We realize that there are many technical and biological issues that need to be overcome prior to execution of the full scale mapping.

### I-50: Embryo Loss Due to Epigenetic Anomalies in the Male Germ Line: Role of Estrogen

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**Background:** To investigate if aberrant methylation and expression of imprinted genes of the Igf2-H19 locus in the spermatozoa and embryos could be a paternal epigenetic factor involved in early embryo loss. To elucidate the role of estrogen in acquisition of the imprinting at the Igf2-H19 locus during spermatogenesis.

**Materials and Methods:** Adult male rats of Holtzman strain were administered tamoxifen citrate at a dose of 0.4mg/kg/day for 60 days. Control males rats were administered water only. Control and tamoxifen treated males were mated with normal cycling females a week before sacrifice. Caudal spermatozoa and normal and resorbed embryos sired by these males were processed for DNA methylation status at the Igf2-H19 imprinting control region (ICR) by Bisulfite sequencing, Methyl Specific PCR and Combined Bisulfite Restriction Analysis. The effect of tamoxifen treatment of global DNA methylation in spermatozoa was studied by methylation mapping of Line 1 elements and 5-methyl cytosine content by flow cytometry. Expression of Igf2 protein and H19 transcript was studied in normal and resorbed embryos. To elucidate the role of estrogen, estrogen response elements (ERE) were identified in the Igf2-H19 ICR (H19 DMR) and its functionality determined in the male germ cells by Chromatin-immunoprecipitation with DNA $\beta$  antibody. In addition, interaction of ER  $\beta$  with Estrogen receptor methyltransferase, Dnmt1 and PCNA in the male germ cells was studied by immunoprecipitation and confocal studies.

**Results:** Methylation analysis by bisulfite sequencing of CpG island at H19 DMR in control and tamoxifen treated male rats showed association between methylation at H19 DMR in the spermatozoa and embryo loss. This observation suggests potential application of H19 DMR methylation in spermatozoa as a predictive factor to assess embryonic development. The study also showed hypomethylation at H19 DMR in spermatozoa upon tamoxifen treatment indicating an effect of tamoxifen in the establishment and/ or maintenance of methyl imprint during spermatogenesis. Since tamoxifen is a selective estrogen receptor modulator, the effects could be mediated through estrogen associated signaling pathways. An indicator of genome wide methylation, Line1 and 5-methyl cytosine content did not show any change with tamoxifen treatment confirming Igf2-H19 locus specific effect of tamoxifen treatment. Combined bisulfite restriction analysis and bisulfite sequencing of

H19 DMR in embryo revealed loss of methylation on the paternal allele in resorbed embryo. Resorbed embryo from control and tamoxifen group showed decrease in Igf2 protein expression and no change in H19 gene expression. This observation indicated deregulation of imprinting of Igf2 in embryo leading to aberrant expression of this gene. Deregulation of imprinting leading to aberrant expression of Igf2 could be one of the factors leading to resorption of embryo. Methylation error on the paternal allele could be responsible for loss of imprinting bringing about aberrant expression of Igf2 leading to embryo resorption. In rat testicular germ at H19 DMR was  $\beta$  cells, a functional estrogen response element binding ER was  $\beta$  detected. Also, a colocalization and interaction between Dnmt1 and ER observed in rat testis. These observations of protein-protein and protein-DNA interaction prompted us to propose a model for DMR methylation. Dnmt1 complexed, bound to ERE on Igf2/H19 locus could catalyze methylation of  $\beta$  with PCNA and ER-ERE  $\beta$  CpG island on H19 DMR. With tamoxifen treatment, reduction in ER interaction was observed which could be attributed to antiestrogenic action of tamoxifen leading to inhibited targeting of PCNA-Dnmt1 complex. This could result into methylation error in spermatozoa either by interfering with maintenance or spreading activity of Dnmt1.

**Conclusion:** The study demonstrates significant hypomethylation at the H19 DMR in the spermatozoa of tamoxifen treated males, which is transmitted to its progeny affecting their developmental potential. The study indicates that methylation at the H19 DMR in the spermatozoa could be used as a predictive factor for pregnancy outcome. The study suggests involvement of estrogen in the acquisition of imprint in the male germ cells and explains the possible impact of environmental estrogens on male reproductive health.

**Keywords:** Genomic Imprinting, Estrogen Signaling, Paternal Factor, Embryo Loss

### I-51: The Role of the Transcription Factor GCNF in Germ Cell Differentiation and Reproduction in Mice

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The germ cell nuclear factor (GCNF) is a member of the nuclear receptor super family of transcription factors. GCNF expression during gastrulation and neurulation is critical for normal embryogenesis in mice. GCNF represses expression of the POU domain transcription factor Oct4 during mouse post-implantation development *in vivo*. Oct4 is thus down-regulated during female gonadal development, when the germ cells enter meiosis, which is a process important for reproduction,

but one that is rare in germ cells derived from embryonic stem cells *in vitro*. One aim of our work is to better define the role of GDNF during mouse germ cell development *in vivo*. We observed a steady decrease in pluripotency-associated gene activity with a concomitant up-regulation of GDNF expression in germ cells derived from developing fetal gonads one day prior to the onset of meiosis. Meiosis-associated genes were then up-regulated at onset of meiosis. These findings suggest that GDNF may repress Oct4 expression in female germ cells and that it plays a role in initiation of meiosis or in activation of meiosis-associated genes in female germ cells. To further investigate the role of GDNF in meiotic processes in male germ cells we generated a GDNF knock-down model to monitor the effect of GDNF during spermatogenesis in a functional manner. The ultimate goal of our studies is to better understand key mechanisms during germ cell development that will serve the *in vitro* derivation of healthy and functional gametes.

### **I-52: Maternal mRNA Metabolism during Oocyte-to-Zygote Transition**

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**Background:** Maternal mRNA degradation is a selective process that occurs in waves corresponding to important developmental transitions such as resumption of meiosis, fertilization and zygotic genome activation. It has been demonstrated that the number, position, and combination of 3' UTR cis-acting elements interacting with trans-acting protein factors regulate translation and mRNA stability. Our goal is to integrate bioinformatic analysis of microarray expression data and experimental analysis of mechanisms underlying control of maternal mRNA degradation.

**Materials and Methods:** Bioinformatic analysis of microarray expression data from oocytes and early embryos, knock-down of candidates regulating maternal mRNA stability, and biochemical analysis of the role of candidates in reporter assays in cell culture.

**Results:** We found that the decapping complex is strongly upregulated during resumption of meiosis. Our analysis of microarray expression data from oocytes has revealed that the length of 3' UTR inversely correlates with mRNA degradation during meiosis. Furthermore, analysis of 3' UTR composition of maternal mRNAs has disclosed that AU-rich motifs are strongly associated with meiotic transcriptome remodelling. While U-rich elements were predominantly present in naturally unstable transcripts in oocytes, several AU-rich motifs were significantly enriched in mRNAs that are rather stable during meiotic maturation. To get more insight into underlying mechanisms, we analyzed expression of >20 genes en-

coding proteins binding AU-rich sequences. We found that Elavl2 expression is enhanced in the oocyte relative to somatic tissues (except of neurons).

ELAVL2 is an excellent candidate for a maternal factor stabilizing maternal mRNAs during meiotic maturation. Effects of the loss of ELAVL2 on the oocyte-to-zygote transition are currently under investigation.

**Conclusion:** Maternal mRNA degradation occurs in phases and the first wave maternal mRNA degradation during meiotic maturation appears to be composed of selective mRNA stabilization during global upregulation of mRNA degradation.

### **I-53: Genetics of Infertility: How to Clone Human Genes Solely Involved in Infertility Phenotype**

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An increased proportion of couples require a medical help to conceive and 1-3.6% of pregnancies in occidental countries are obtained thanks to a Assistance Reproduction. For more than half of them the cause of these dysfunctions remains unknown and *in vitro* fertilization is often proposed as a universal answer to a complex problem. Most of the proposed treatments are often empirical and little has been achieved to better understand the mechanisms underlying human fertility. This is not conforming to a medical practice of 2010.

Little has been discovered in the field of male reproductive genetics since the discovery of chromosome Y microdeletions. Many groups looked in various cohorts of infertile patients for causal mutations in candidate genes. This strategy has mainly been unsuccessful probably mainly because of the tremendous genetic heterogeneity of this pathology. We recently demonstrated that whole genome homozygosity mapping applied to appropriate families or group of patients could provide high quality results (ref). This strategy, which highlights the presence of identical ancestral chromosomal regions is particularly suited to the study of large consanguineous families or inbred communities.

In collaboration with centres in France, but especially in North Africa and the Middle East we have started recruiting patients with well defined phenotypes. These patients often come from small communities with little or no immigration and a high degree of intra-familial marriage. Husband and wife are thus often related and susceptible to harbour the same rare dormant recessive mutation they have inherited from a common ancestor. Their children thus have a risk of ¼ to inherit the two copies of the mutation, thus to develop the syndrome. Since the mutation comes from a recent common ancestor the whole region surrounding the mutation will

be identical, therefore all the polymorphic markers of the area will be homozygous. In these situations, when the phenotype is homogeneous and the families inbred, the strategy of homozygosity mapping allows the identification of the regions likely to hold the mutation responsible for the investigated phenotypes.

We have used this approach successfully in the past and believe that this strategy is likely to succeed in identifying many more genes involved in the reproductive process. I will present our latest results in this field. Indeed, we have identified new genes solely involved in male infertility. I will also make a call for collaborations in this specific area of research.

### **I-54: New Models for Human and Mouse Genetic**

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The possibility to reprogram somatic human cells will greatly and deeply change genetic approach and allow the development of new tools to study genetics diseases. Indeed, our ability to study human genetic diseases suffers from the lack of valid *in vitro* models. The latter should (i) be originating from human primary cells, (ii) be able to self-renew for a long time and (iii) be able to differentiate into a wide range of different tissue types. Human embryonic stem (hES) cells harbour all those properties and may provide a first source for establishing such *in vitro* models. Alternatively, reprogramming strategies offer a very new possibility to derive induced-pluripotent stem (iPS) cells from clinically described patients or from complex mouse transgenic models.

Human ES cells carrying genetic disorders are a promising tool to develop *in vitro* cellular model for physiopathology investigations and drug screening. This field is actively growing but suffers of the low number of available embryos.

Ideally to overcome this limitation, cells derived from patients should be reprogrammed to acquire an ES-like phenotype allowing their amplification in an unaltered state and their *in vitro* differentiation in a broad variety of phenotypes. In that context, reprogramming adult cells has focused scientific community interest for their potentially inexhaustible source of cells for both therapy replacement and models developments.

First publication in 2006, Yamanaka's team published the reprogramming of mouse fibroblast toward an ES-like phenotype. Authors identified a minimal set of genes, i.e. Oct3/4, Sox2, c-Myc and Klf4 able to successfully reprogram embryonic and adult fibroblasts into ES-like cells. Reprogrammed cells (called iPS cells, for induced-Pluripotent Stem cells) share ES cells markers and capacities.

With the goal to develop new models for monogenic diseases, we set up a first program based on hESC derivation from affected preimplantation genetic diagnosis (PGD)-identified embryos. So far we have established 17 hES lines for 10 different pathologies. All the cell lines are banked by our collaborator I-Stem. All our cell lines are registered to the European Human Embryonic Stem Cell Registry and available to the scientific community. This represents one of the most important collections of hES cell lines carrying mutation available for the scientific community.

For pathologies not tested by PGD, we implemented somatic cell reprogramming technology to derive iPS from human/mouse fibroblasts. As a first example, we established iPSC from 2 adult patients carrying Friedreich ataxia. Such cells are now under characterisation. In addition, we have established iPS cell lines from different transgenic mouse lines, which are used now either to study their differentiation properties *in vitro* or as a tool to decipher pluripotency.

**Keywords:** Pluripotency, Induced Pluripotent Stem Cells, Monogenic Diseases

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## **Reproductive Imaging**

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### **I-55: Molecular Imaging Overview**

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Molecular imaging is the noninvasive visualization of normal as well as abnormal cellular processes at a molecular or genetic level of function. It is used to provide characterization and measurement of biological processes in living animals and humans (*in vivo*).

The discipline of molecular imaging evolved rapidly over the past decade through the integration of cell biology, molecular biology and diagnostic imaging.

A key component is the imaging probe which homes in on the specific target of interest in the body and is visualized by a special scanning method. In creating a probe the basic principle is to identify a specific receptor site associated with the target molecule

that characterizes the disease process being studied.

The agent is labeled with a radioactive substance or nanoparticle or other methodology that allows detection by the imaging device.

Although the concept is simple, the process is complex requiring extensive expertise and equipment. The probe must be safe, not alter the disease process being studied, be able to reach the target in sufficient concentration while not accumulating in other tissues, and be retained long enough to be detected – all significant challenges to overcome. Currently there are over 500 probes with

many more in development.

Three different noninvasive, *in vivo* imaging technologies are evolving at the heart of molecular imaging to provide spatial and temporal dimensions of understanding:

1. Radionuclide Imaging
2. Magnetic Resonance Imaging (MRI)
3. Optical Imaging

Molecular imaging has two basic applications:

1. Diagnostic imaging to determine the location and extent of targeted molecules for the disease being studied
2. Therapy to treat specific disease-target molecules by adding a therapeutic agent onto the probe.

Molecular imaging will become more important as genomics and proteomics expand the number of relevant molecules to visualize.

We will present an overview of current application in MRI field to be used in Royan project hopefully.

### **I-56: Value of 3D XI (Computed Ultrasound Tomography) in the Evaluation of Benign Prostatic Hyperplasia and Preservation of Men Infertility**

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The prevalence and incidence of clinical problems secondary to and associated with benign prostatic hyperplasia (BPH) have been increasing as the population ages. Men with lower urinary tract obstruction (LUTS) may have sexual dysfunction, including ejaculatory loss, painful ejaculation, and sometimes erectile dysfunction (ED). Even though, the pathogenic relationship between BPH and these symptoms is not completely understood. On the other hand, patients after TURP have a common complaint of retrograde ejaculation.

Many reports have been produced by Watanabe (1998) and Ansari et al. (2000) describing the role of the surgical capsule as the main responsible for outlet stenosis and consequently the severity of LUTS.

However, by 3D XI, transrectal computed ultrasound tomography (TRCUT) could allow for zonal description of the prostatic gland and consequently the accurate appreciation of BPH.

By this technology, the "balance Principle" could be derived (Published in the international journal of urology, 2008), explaining the main reasons for BPH symptoms severity. Characterization of the container within the surgical capsule showed that the BPH nodular balance and type alongside the prostatic urethra are the main factors that impulse and responsible for symptoms severity. In other words, the clinical symptoms of BPH are primarily dependent upon the nodular imbalance effect upon the prostatic urethra.

This principle has proved valuable in therapeutic purpose; to regain the urethral midline balance via TRCUT

guided trans-perineal injection of the BPH nodules.

Our aim is to appraise the value of 3D XI in the evaluation of prostates of aging men and accurately manage the less-invasive treatment for BPH.

In other words to avoid retrograde ejaculation, trans-perineal injection of BPH nodules can preserve the male fertility and correct the balance alongside the prostatic urethra, preserving efficient antegrade ejaculation.

### **I-57: Embolization of Varicocele**

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### **I-58: Sclerosing of Ovarian Cysts**

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### **I-59: Diagnostic Accuracy of Physical Examination, Transvaginal Sonography, Rectal Endoscopic Sonography, and Magnetic Resonance Imaging to Diagnose Deep Infiltrating Endometriosis**

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**Background:** To compare the value of physical examination, transvaginal sonography (TVS), rectal endoscopic sonography (RES), and magnetic resonance imaging (MRI) for the assessment of different locations of deep infiltrating endometriosis (DIE).

**Materials and Methods:** Ninety-two consecutive patients with clinical evidence of pelvic endometriosis. Physical examination, TVS, RES, and MRI, performed preoperatively. Descriptive statistics, calculation of likelihood ratios (LR+ LR-) of physical examination, TVS, RES, and MRI for DIE in specific locations confirmed by surgery/histology.

**Results:** The sensitivity and LR+ and LR- values of physical examination, TVS, RES, and MRI were, respectively, 73.5%, 3.3, and 0.34, 78.3%, 2.34, and 0.32, 48.2%, 0.86, and 1.16, and 84.4%, 7.59, and 0.18 for uterosacral ligament endometriosis; 50%, 3.88, and 0.57, 46.7%, 9.64, and 0.56, 6.7%, -, and 0.93, and 80%, 5.51, and 0.23 for vaginal endometriosis; and

46%, 1.67, and 0.75, 93.6%, -, and 0.06, 88.9%, 12.89, and 0.12, and 87.3%, 12.66, and 0.14 for intestinal endometriosis.

**Conclusion:** The MRI performs similarly to TVS and RES for the diagnosis of intestinal endometriosis but has higher sensitivity and likelihood ratios for uterosacral ligament and vaginal endometriosis.

**Keywords:** Endometriosis, Deep Infiltrating Endometriosis, Ultrasonography, Magnetic Resonance Imaging, Comparative Studies, Laparoscopy

### **I-60: Sonographic Evaluation of the Fetal Skeletal Anomalies**

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### **I-61: Second Trimester Genetic Sonography**

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# Oral Presentations

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## Andrology

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### O-1: Impaired Reproductive Parameters of Male Rats Infected with *Toxoplasma Gondii*

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**Background:** *Toxoplasma gondii* is a protozoan parasite that infects up to a third of the world's population. Toxoplasmosis is one of the classical conditions known to have a profound adverse effect on human reproductive functions, but a few investigations on male reproductive parameters were done. The aim of this investigation is Study on the effects of *Toxoplasma gondii* on reproductive parameters in male rats.

**Materials and Methods:** A total of 56 male rats divided into infected group (IG) and control group (CG). The RH strain of *T. gondii* tachyzoites were injected interaperitoneally in an infected group of 35 rats, while 21 rats were used as controls. On days 10, 20, 30, 40, 50, 60 and 70 post-injection (PI), 5 rats from infected group and 3 rats from control group were anesthetized. The percentage of body weight to testis weight ratio (BTR) as well as epididymal sperm parameters (motility, viability, number and morphology) was investigated.

**Results:** BTR was not significantly change in IG and CG groups on days of 10 to 70 PI ( $P \geq 0.05$ ). Sperm motility was significantly decreased on days 10, 20, 30, 40, 50, 60 and 70 PI ( $p < 0.05-0.01$ ). Sperm viability was significantly decreased on days 10, 20, 30, 40, 50 and 60 PI ( $p < 0.05-0.01$ ). Sperm concentration was significantly decreased on days 20, 30, 40, 50 and 60 PI ( $p < 0.05-0.01$ ). A sperm abnormality was increased on days 30, 40 and 50 PI ( $p < 0.05-0.01$ ).

**Conclusion:** According to the results, toxoplasmosis can cause impaired on the reproductive parameters of male rats.

**Keywords:** Reproductive Parameters, Male Rats, *Toxoplasma Gondii*, Toxoplasmosis

### O-2: Prospective Evaluation of the Threat Related to the Use of Seminal Fractions from Hepatitis C Virus-Infected Men in Assisted Reproductive Techniques

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**Background:** The risk of hepatitis C virus (HCV) transmission during assisted reproductive techniques (ARTs) is still disputed and no report concerning its prospective evaluation is available. The aim of this 4-year follow-up multicentre study that enrolled 86 HCV-serodiscordant couples was to determine whether a sperm-processing method was able to reduce levels of HCV in semen and the risk of HCV transmission to the newborn.

**Materials and Methods:** All the men were chronically infected by HCV and 10 of them by human immunodeficiency virus. A total of 181 seminal plasmas and 153 sperm fractions were tested for the presence of HCV RNA.

**Results:** HCV RNA tested positive in 20.4% of the seminal samples. All of the 153 final sperm fractions tested negative for HCV. The detection of HCV RNA in semen was significantly correlated with a high viral load in blood ( $p < 0.05$ ). The presence of HCV RNA in seminal plasma impaired neither semen parameters nor ART issue. From the 58 couples enrolled effectively in an ART programme, 24 pregnancies and 28 newborns were obtained. All of them tested negative for HCV RNA in blood.

**Conclusion:** These results emphasize the safety of the semen-processing method. The negligible risk of transmitting HCV reduces the value of the systematic analysis of HCV RNA in seminal fractions prior to ART. Since use of this analytical procedure involves the freezing of semen, its avoidance would result in an increase in sperm quality and reduce the need to perform intracytoplasmic sperm injection techniques

**Keywords:** Assisted Reproductive Techniques, Hepatitis C, Infertility, Viral Threat

### O-3: In-Situ Preservation of Caprine Cauda Epididymal Spermatozoa at -10 Degree Centigrade

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**Background:** In-situ preservation of cauda epididymal spermatozoa at -10 degree centigrade with electrolyte free media for obtaining maximum functional gametes than preservation at 5 degree centigrade.

**Materials and Methods:** Electrolyte free media prepared with soybean lecithin-glycerol, Coenzyme Q10 - glycerol and soybean lecithin - Coenzyme Q10-glycerol were inoculated separately into ligated cauda epididymides, equilibrated 2 h at 5 degree centigrade,

wrapped with aluminium foils and freezed at - 10 degree centigrade. Spermatozoan characters were evaluated 7 and 21 days after thawing at 38.5 0C in a water bath for 5 min.

**Results:** Spermatozoan characters were diminished gradually and significantly ( $p < 0.001$ ,  $p < 0.05$ ) between the media and observation days. Soybean lecithin-Coenzyme Q10-glycerol effectively protected spermatozoa against cold shock where spermatozoan progressive motility, livability, hypo-osmotic swelling positivity were  $30.2 \pm 0.62$ ;  $45.2 \pm 0.82$  and  $41.6 \pm 0.79$  percent respectively on day 21.

**Conclusion:** This method can be adopted in field conditions for transportation of frozen epididymides and reutilization of maximum functional gametes to conserve valuable animals after death/ postmortem / slaughter.

**Keywords:** Coenzyme Q10, Electrolyte Free Medium, In-Situ Epididymal Spermatozoa, Freezing, Hypo-Osmotic Swelling Test

#### **O-4: Effects of Fatty Acid Sources in Diet on Sperm Characteristics of Iranian Ram**

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**Background:** Researchers have indicated that mammalian spermatozoa are characterized by a high proportion of saturated, n-6 and n-3 series of fatty acids (FA). Major FA in rams' sperm consists of C16, C18:1 and C22:6 (DHA). Little information exists on the effective use of saturated and unsaturated FA for ram whereas lipids have important role in sperm fertility. Our Background was to determine effects of feeding diets containing saturated or unsaturated FA sources influences ram's sperm quality during 12 weeks in breeding season.

**Materials and Methods:** Nine 2.5 years old fat-tailed Iranian rams (Kalkohi) were randomly assigned to 3 groups from Sep. - Dec. 2009. The treatments were diets containing (35 g/ d/ ram) RP-10® (C16:0 source), sunflower oil (SO) (C18:2 source) and fish oil (FO) (n-3 source) with a constant level of Vitamin E. The experiment had 16 weeks, with the first 4 weeks for diet adaptation and the last 12 weeks for collecting semen once a week by artificial vagina (AV). The volume of the collected semen was measured and sperm motility was assessed using a light microscope. Sperm concentration was estimated microscopically using a Neubauer® counting chamber and sperm viability was measured by the eosin-nigrosin

staining method. Data were analyzed using the GLM procedure of SPSS 11.

**Results:** Positive responses to added FO was seen after 4th weeks of data collection and it was continue during sampling weeks. Various treatments have been changed semen volume of experimental rams. Interestingly, FO had the highest total sperm output versus RP10 and SO (4.3, 4.6. and  $5.2 \times 10^9$  for RP10, SO and FO respectively;  $p < 0.05$ ). Total motility (72, 74 and 78 % for RP10, SO and FO respectively;  $p < 0.05$ ) and progressive motility (51, 54.5 and 60 % for RP10, SO and FO respectively;  $p < 0.05$ ) as well as viability (75, 77.5 and 81 % for RP10, SO and FO respectively;  $p < 0.05$ ) were influenced by treatments.

**Conclusion:** This is the first study on use C16:0 FA source in ram diet. It appears that this fatty acid cannot improve sperm quality such as unsaturated FA. Previous reports alongside our data suggest that an n-3 FA source effect rams sperm parameters. Our results suggest that FO can be effectively evaluated useful sperm parameters which they can improve fertility.

**Keywords:** Ram, Sperm, Diet Fatty Acid

#### **O-5: Identification of Novel Immunodominant Epididymal Sperm Proteins Using Combinatorial Approach**

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**Background:** Alteration in the protein signatures of functionally immature testicular spermatozoa occurs during their journey through the epididymis. This leads to acquisition of sperm domain specific functions essential for successful fertilization. Epididymal sperm proteins are preferred targets for immunocontraception as well as in elucidating the causes of infertility. The Background of the study was to identify epididymis-specific proteins in different domains of the sperm which could be exploited as putative targets for the development of post testicular male contraceptives.

**Materials and Methods:** We used a novel combinatorial approach comprising of modified subtractive immunization (SI), followed by proteomics. Neonatal mice were tolerized on day 0 and day 5 with testicular proteins and immunized in adulthood with proteins of the epididymal sperm domains, i.e. intact and soluble fractions of head and flagella (HI/ HS and FI/ FS). Post immune sera (PI) were characterized by ELISA, Indirect immunofluorescence (IIF), Immunohistochemistry and western blotting. Immunoreactive proteins were sequenced using mass spectrometry. Validation of novel proteins was

performed by peptide ELISA.

**Results:** ELISA indicates that the animals were tolerized to testicular proteins and upon challenge with the immunogen showed immune response specifically to epididymal proteins. IIF results indicate that post immune sera localized antigens specifically in the sperm regions with which they were immunized. Immunohistochemistry, using PI sera of all the four groups demonstrated intense reactivity starting from distal caput with three of the four sera (HI, HS and FI) and proximal caput with the serum of the fourth group (FS). Post immune sera were used for immunoproteomics which led to identification of 30 proteins, of which four proteins namely Sperm Head protein 1(SHP1) (Accession no. P85300), Sperm Flagella protein 2 (SFP2) (Accession no. P85301), Sperm Flagella protein 3 (SFP3) (Accession no. Q5BK63) and Sperm Flagella protein 4 (SFP4) (Accession no. Q06647) are being reported for the first time on sperm. Another group of four proteins namely Collagen alpha-2 (I) chain precursor, Homeodomain interacting protein kinase1, GTP binding protein Rab1 and Ubiquinol cytochrome c reductase core protein II although reported earlier in testis are being reported for the first time in epididymal sperm. The possibility that the proteomics data represented abundant comigrating proteins instead of the immunoreactive group of proteins was ruled out by validation of the seven out of the eight novel proteins by peptide ELISA.

**Conclusion:** The unique strategy helped in identifying number of new epididymal proteins on distinctly different domains of the sperm predominantly on the flagella. These proteins are immunogenic in nature, conserved in rodents. These data are a useful repository which could be exploited to develop targets for post testicular immunocontraception or biomarkers for infertility diagnosis and management.

**Keywords:** Sperm Protein, Epididymis, Head, Flagella, Proteomics

### **O-6: Response to Potassium Paraaminobenzoate (Potaba<sup>TM</sup>) or Iontophoresis with Verapamil and Dexamethasone (Physion<sup>TM</sup>) in Peyronie's Disease**

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**Background:** Effectiveness of Treatment

**Materials and Methods:** Patients with a diagnosis of PD were offered Potaba for 6 to 18 months or 18 applications of Physion for 6 weeks. They were requested to complete a questionnaire before and after treatment.

The data was collected from December 2004 to November 2009 and analysed using SPSS version 12 for Windows to evaluate the effectiveness of treatments.

**Results:** Presenting complaint (80%) followed by erectile dysfunction (30%), pain (30%) and lump (21%). Only 34/178 (19%) patients who received Potaba had completed the post treatment questionnaire. 25 of these 34 (74%) patients had a stable plaque for at least 6 months and 16/34 (47%) reported a benefit after treatment with Potaba. Patients reported these benefits: Reduction in bend (7 individuals), removed bend (2), lowered pain (1), more feeling at intercourse (1), stronger erection (2). All 17 patients who received Physion had completed the post treatment questionnaire. 14 of these 17 (82%) patients had a stable plaque and 13/17 (76%) reported a benefit from Physion. Patients reported these benefits: Reduction in bend (7 individuals), removed bend (1), lowered pain (3), soften lump (1), stronger erection (1). None reported any worsening of symptoms or serious side effects from either of the treatments.

**Conclusion:** A significant proportion of patients who received Physion for PD reported benefits from the treatment unlike Potaba. None reported any adverse effects. However this should be interpreted cautiously in view of small numbers of patients on Physion and low response rate for completion of questionnaire post Potaba treatment.

**Keywords:** Peyronie's, Physion, Potaba

### **O-7: Effect of 830 nm Diode Laser Irradiation on Human Sperm Motility**

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**Background:** Sperm motility is known as an effective factor in male fertility and it depends on energy consumption. Low level laser irradiation could increase energy supply to the cell by producing of adenosine triphosphate (ATP). The purpose of this study is to evaluate how the laser irradiation affects the human sperm motility.

**Materials and Methods:** 22 Human semen samples were used in this study. Each sample was divided into 4 equal portions and irradiated by 830nm GaAlAs laser irradiation with varying doses as: 0 (control), 4, 6 and 10 J/Cm<sup>2</sup>. At the times of 0, 30, 45 and 60 minutes following irradiation, sperm motilities are assessed by means of computer-aided sperm analysis (CASA) in all samples. Two additional tests (HOS and SCD tests) also performed on the control and high irradiated groups

as well.

**Results:** In control groups, progressive motility significantly decreased by passing of time, while those of irradiated groups remained constant or significantly increased in doses of 4 and 6 J/cm<sup>2</sup>, at the times of 60 and 45 min, respectively. At the time of 30, progressive motility significantly increased in dose of 10, while at time of 45, it significantly increased in doses of 4 and 6, and at time of 60, it significantly increased in all three doses, in comparison with control groups.

**Conclusion:** These results suggest that irradiating human sperms with 830nm diode laser at 4, 6 and 10 J/cm<sup>2</sup> energy density doses can improve their progressive motility which may be related to increasing of energetic efficiency. The maximum effect appears on doses of 4 and 6 J/cm<sup>2</sup>, and at the times of 45 and 60 minutes after irradiation.

**Keywords:** Sperm Motility, Laser Irradiation

### O-8: The Effect of Body Mass Index on Semen Quality and Reproductive Hormones

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**Background:** Recent population-based studies suggest an elevated risk for subfertility among couples in which the male partner is obese and an increased likelihood of abnormal semen parameters among heavier men. Male factor infertility is associated with a higher incidence of obesity in the male partner. The aim of this review article is to study the effect of body mass index (BMI) on semen quality and reproductive hormones.

**Materials and Methods:** This review article prepared by studying of more than 20 articles obtained from Google, pub med sites with key words such as: Obesity; male infertility; sperm parameters; oligozoospermia; reproductive hormones; estrogen; testosterone

**Results:** Anette S. Aggerholm et al. (2009) reported that T and inhibin B serum concentrations were 25%-32% lower in obese men in comparison with normal-weight men, whereas the E2 concentration was 6% higher in obese men. Overweight men (BMI, 25.1-30.0 kg/m<sup>2</sup>) had a slightly lower adjusted sperm concentration and total sperm count than did men with a normal BMI (20.0-25.0 kg/m<sup>2</sup>), but no reduction in sperm count was observed among the obese men. Floor H. Duits et al. demonstrated the group of men with a BMI > 30 kg/m<sup>2</sup> had a lower semen volume compared with the group with a BMI between 20 and 25 kg/m<sup>2</sup>. Carlsen et al. (2008) found that free androgen index and E2 increased with increasing BMI and Percentages of normal spermatozoa were reduced, although not significantly, among men with high BMI. Ahmad O. Hammoud et al. (2008) found that The incidence of oligozoospermia increased with increasing BMI: normal weight = 5.32%, overweight = 9.52%, and obese = 15.62%. The prevalence of a low

progressively motile sperm count was also greater with increasing BMI: normal weight = 4.52%, overweight = 8.93%, and obese = 13.28%. The incidence of erectile dysfunction did not vary across BMI categories when corrected for potential contributing factors. Jorge E et al. (2009) reported that Sperm with high DNA damage were significantly more numerous in obese men than in normal-weight men.

**Conclusion:** The increasing prevalence of obesity calls for greater clinician awareness of its effects on fertility, better understanding of underlying mechanisms, and eventually avenues for mitigation or treatment.

**Keywords:** Obesity, Sperm Parameters, Reproductive Hormones

### O-9: Exercise and Supraphysiological Dose of Nandrolone Decanoate Increase Apoptosis in Spermatogenic Cells

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**Background:** Anabolic-androgenic steroids (AAS) are used at high doses by athletes for improving athletic ability, physical appearance and muscle mass. Unfortunately, the abuse of these agents has significantly increased. It has been established that exercise and high doses of AAS may influence the hypothalamic-pituitary gonadal (H-P-G) axis, which can in turn affect the testicular apoptosis. However, the effect of the combination of exercise and high dose of AAS on testicular apoptosis is not known. This study has investigated the combined effects of exercise and high doses of nandrolone decanoate on apoptosis in the spermatogenic cell lineage.

**Materials and Methods:** Five groups of male Wistar Strain albino rats were treated as follows for 8 weeks: solvent of nandrolone decanoate (peanut oil) as a vehicle (Sham); nandrolone decanoate (ND) (10 mg/kg/weekly) (ND); exercise (1 hr/day, 5 days a week) (exercise); ND (10 mg/kg/weekly) and exercise (1 hr/day, 5 days a week) (ND-exercise); and sedentary control without any injection or exercise (Control). Apoptosis in the male germ line was characterized by TUNEL,

caspase-3 assay and transmission electron microscopy (TEM).

**Results:** The weights of the testis and accessory sex organs, as well as sperm parameters significantly decreased in the experimental groups relative to the sham and control groups ( $p \leq 0.05$ ). Germ cell apoptosis and a significant decrease in the number of germ cell layers in ND-exercise treated testes were observed ( $p \leq 0.05$ ).

**Conclusion:** Exercise training seems to increase the extent of apoptotic changes caused by supraphysiological dose of ND in rats, which in turn affects fertility.

**Keywords:** Apoptosis, Exercise, Nandrolone Decanoate, Spermatogenesis

### **O-10: PLAP Marker in Testicular Biopsy of Iranian Men by Immunohistochemistry Method for Early Diagnosis of Testicular Carcinoma in Situ**

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**Background:** Testicular Carcinoma in situ (CIS), also known as intratubular germ cell neoplasia (ITGCN), is a pre-invasive precursor of testicular germ cell tumours (TCGTs), i.e seminomas and non-seminomas -the commonest cancer type of male adolescents and young adults- which can be diagnosed using a surgical biopsy. Infertility is one of the condition known to predispose to TGCT, but based on scarce existing data, the prevalence of CIS in this risk group was estimated approximately 1% (in range of 0-3.5%) in 15-35 year men in the world. In this study, we investigated the prevalence of CIS based on testicular biopsies performed for infertility reasons in group of males in Avicenna Research Institute.

**Materials and Methods:** There were 722 biopsies during 1386-1388 evaluated morphologically then 88 of cases were evaluated by immunohistochemistry for placental-like alkaline phosphatase (PLAP) -the known established markers for CIS- by an experienced pathologist.

**Results:** CIS was detected in 5 individuals, whom all of them were unilateral and 2 of cases were whole testis. In this study we have prevalence of 0.69% (95% CI 0.21-1.17%) in infertile males.

**Conclusion:** This is the first study in Iran, in future we wish to be able to assess future trends in CIS incidence rates according to increasing incidence rate in industrial countries.

**Keywords:** Testicular Carcinoma in Situ (CIS), PLAP Marker, Immunohistochemistry, Infertility

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## **Embryology**

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### **O-11: Prenatal Oogenesis: Selecting the Quality and Quantity of Oocytes in the Ovarian Reserve**

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**Background:** The purpose of this research programme is to improve understanding of the molecular and cellular processes that lead to selection of oocytes before birth. Vastly more oocytes are produced prenatally than can be utilised in reproductive life. Around 70%, of oocytes formed are eliminated before birth and never contribute to the ovarian reserve. After birth, the number of oocytes continues to decline throughout life to the menopause. Which oocytes are selected for deletion and why? This question remains enigmatic. With such powerful selection, one might expect that only the most perfect oocytes, with the highest capacity for development, will survive. However, this is clearly not the case because many oocytes that reach ovulation and fertilisation are abnormal and human fertility is often suboptimal. Therefore, our research aims to understand the criteria by which oocytes are selected for contribution to the ovarian reserve. A number of approaches and papers spanning over a decade have helped to elucidate some of the answers.

**Materials and Methods:** Human fetal ovaries are collected by collaboration with organisations undertaking termination of pregnancy in the second trimester. There is no contact between researchers and patients. Patients are given information about the study by a member of their healthcare team and invited to participate, without any effect upon their treatment, regardless of their decision. If patients consent, ovaries are removed from fetuses as soon as possible after delivery and taken to the laboratory. In view of the limited supply of human material for research, mouse fetal ovaries are also utilised, from timed matings, to ensure precise dating of pregnancies. On occasion, specific mouse genotypes are also used, for example in our work on p53 knock-out mice. Ovaries are examined by a range of methods, specifically including (1) immunocytochemistry, to unequivocally identify the stages of meiotic prophase I or particular molecular configurations such as recombination foci, (2) FISH, to identify individual chromosomes, (3) tissue culture, with a range of environmental conditions and supplements, to identify which oocytes survive and to optimise conditions for their study, (4) molecular markers of apoptosis, to identify oocytes that have been selected for removal from the viable pool.

**Results:** Our results have shown that oocyte selection for apoptosis includes, but is not restricted to those with genetic abnormalities, since apoptotic markers do not overlap 100% with degenerating oocytes. We have

shown that oocytes can survive *in vitro* under a range of conditions, but that progression beyond the pachytene stage of meiotic prophase I is rarely observed. We have shown that the timing of entry into meiotic prophase I, and the timing of progression through it, may affect likelihood of survival, and that p53 status in the mouse mother or the fetus may affect oocyte formation.

**Conclusion:** Apoptotic markers and cytogenetic abnormalities are both indicative of oocyte lack of viability, however, these are not coincident and environmental factors also contribute to oocyte survival or selection for removal from the pool.

**Keywords:** Oocyte, Fetus, Oogenesis, Prenatal, Apoptosis

### O-12: Study of Expression of Developmental Genes in SCNT Cloned Embryos

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**Background:** 1. To produce somatic cell nuclear transfer (SCNT) embryos of buffaloes. 2. To study gene expression profile of important developmental genes at different stages of SCNT cloned embryo. 3. To study epigenetic reprogramming during early developments of SCNT embryos

**Materials and Methods:** Expression analysis of developmental genes was done in different (ovarian granulosa and cumulus and skin fibroblasts) donor cells; *in vitro* maturing oocytes and different stages of developing SCNT cloned embryos. IVF embryos were kept as controls. Effect of various media, hormones and other supplements at different levels was studied on SCNT embryos production rates and their related gene expression profile. Cultures were developed from different donor tissues and passaged serially. Primary oocytes from abattoir buffalo ovaries were matured *in vitro*. IVM oocytes were enucleated using micro manipulators and one cell was transferred as nuclear donor. Cell-oocyte complex was stimulated using two DC pulses using ECM-2001 and chemically activated by using cycloheximide and then cultured in TCM-199. The c-DNA was prepared from donor cells, oocytes and developing embryos (SCNT and IVF) using a cell to cDNA kit (Ambion). Real-time PCR primers were designed using BEACON DESIGNER and Q-PCR was performed on Mx3000p (Stratagene) using SYBR Green supermix. Data on mRNA expression were analysed using light cycler software to determine differences in gene expression pattern.

**Results:** Expression of chromatin remodelling mRNAs; HDAC1, DNMT1, DNMT3a and DNMT3b was checked at various passages of skin fibroblast, cumulus and granulosa cell lines. HDAC1 mRNA expression was significantly lower in cumulus cells than skin fibroblast and granulosa cells. DNMT1 mRNA expression was sig-

nificantly higher in cumulus cells than other cell types. DNMT3a expression was lower in granulosa cells than other cells. DNMT3b expression was higher in cumulus cells. Thus cumulus cells showed better expression of remodelling genes than other donor cells. The mRNA expressions of GAPDH (reference), Cx43, GDF-9, FGF-4 and Fibronectin were studied to evaluate effects of epigenetic modification by lectin supplementation during oocyte maturation. Relative mRNA abundance of all the four genes was significantly higher in 10 µg/ml lectin dose in comparison to other treatment groups, showing utility of lectins in IVM of oocytes. IGF-1 and IGF-2 mRNA transcript level was significantly higher in NT embryos developed from different cell types than IVF embryos. IGF-1R and IGF-2R mRNA transcript level from cumulus cells was comparable to IVF. The embryo production rate of SCNT buffalo from granulosa and skin fibroblasts was significantly lower than from cumulus cells and IVF.

**Conclusion:** Our results indicate that cumulus cells are best nuclear donor for SCNT clone production because of their better reprogramming capabilities. The mRNA transcript levels of developmental genes were significantly altered by culture medium and media supplements. There is a possibility of improving gene expression and efficiency of SCNT cloned embryos production by epigenetic modification at different stages for developing ES cells from respective donor for therapeutic applications.

**Keywords:** Gene Expression, IVM, SCNT Embryo, QPCR

### O-13: Exogen Melatonin Protects Spermatogenic Cells from Apoptosis in Mouse under Chemotherapy

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**Background:** The aim of this study was to investigate the possible protective role of melatonin on the apoptosis of germ cells in chemotherapy-induced spermiotoxicity.

**Materials and Methods:** Male adult NMRI mice were divided into four groups. Then each group were divided into two subgroups of a and b. Group 1 (control): mice received vehicle (ethanol 1%) for 5 days; Group2 (Busulfan): mice received a single dose of 20 mg/kg busulfan. Group 3 (Melatonin) mice: received melatonin (10 mg/kg) for 5 days. Group 4 (Busulfan+Melatonin): received a 5-day course of melatonin (10 mg/kg) following an initial dose of busulfan (20 mg/kg). Evaluations were made using *in situ* TUNEL assay after 5 days (a-

subgroup) or 35 days(b-subgroup) and assay of plasma testosterone.

**Results:** Busulfan-treated mice both in a and b-subgroups, showed a significant increase in the numbers of apoptotic cells ( $p < 0.01$ ) compared to controls. Melatonin in group4 significantly reduced rate of apoptosis however it was not effective on apoptotic cells in group 3. A significant decrease in testosterone level was observed in group2 (subgroups of a and b) ( $p < 0.01$ ). Administration of melatonin in group4 (subgroups of a and b) significantly increased the levels of testosterone and caused to normalization in testosterone levels compared to group2 ( $p < 0.05$ ).

**Conclusion:** These results indicated that melatonin may have a protective effect against busulfan-induced testicular damage, partly by decreasing of apoptosis and alteration in pituitary gonadal axis

**Keywords:** Apoptosis, Busulfan, Melatonin, Testis

### **O-14: New Era in Sperm Selection for ICSI Procedure**

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**Background:** Intra-cytoplasmic sperm insemination, the ICSI, is considered as the one most routine treatment of male infertility. Although the genetic inheritance of an individual is based on the genetic contribution from both the oocyte and sperm, considering the fact that ICSI by passes all the barriers to natural fertilization much emphasis was given to oocyte quality to improve the ICSI outcomes. However, recent studies suggest that sperm quality has a profound influence on ICSI outcomes and selection of sperm based on functional characteristics along with morphology and motility may significantly improve fertilization, embryo quality, implantation, and take home baby rates in ICSI. These new sperm selection procedures has led to "New era in sperm selection procedure" which are different or complementary to routine sperm processing techniques such as "swim up or density gradient centrifugation procedure". In order to establish such techniques comprehensive understanding of sperm functions including: sperm membrane functional characteristics, sperm surface proteins, and sperm DNA chromatin integrity and maturity, are essential.

**Materials and Methods:** In the first part of the study, we implemented the sperm functional characteristics test, then the relation of these test with semen parameters, and ICSI outcomes including fertilization, embryonic stage and quality, implantation and pregnancy rates were obtained. In the second part of the study different sperm characteristics upon which sperm can be selected including the sperm surface charge, the Zeta potential and the sperm surface receptor, the hyaluronic acid (HA) receptor were studied and functional characteristic

of sperm selected in these procedures was compared to their control group, in order to confirm the efficiency of these techniques. In the third part, once the efficiency of each technique was confirmed they were implemented for ICSI procedure in two different trails.

**Results:** The results, the first part of the study, showed that assessment of protamine deficiency, chromatin integrity by CMA3 staining, sperm chromatin dispersion test, tunnel, and comet assay show significant correlation with semen quality and ICSI outcomes. Thus suggesting these tests are suitable for assessment of sperm functional characteristics. Then, the efficiency of Zeta and HA sperm selection procedure based on previous studies were evaluated and the results, of the second part of the study, showed that both techniques are efficient to select normal sperm in term of sperm morphology, normal protamine content and DNA integrity, however, Zeta procedure was more efficient for selecting sperm with DNA integrity while the HA method can more efficiently select sperm with normal morphology. Following confirmation of the efficiency of these two Novel procedures we implemented these two procedures in ICSI trails along with the appropriate controls. The third part of the study, these trials showed that both techniques significantly improve fertilization rate but in our trails only the Zeta procedure significantly improved implantation and pregnancy rates. In a single case study we implemented this procedure for couple with 11 failed IVF/ICSI cycles which resulted in successful pregnancy. Further trails, including multicenter trails, are also being carried out to further verify the efficiency of the Zeta procedure on ICSI outcome.

**Conclusion:** The results of these studies, along with complementary studies in the literature suggest that the novel sperm selection procedure for ICSI should become gradually mandatory for implementation of ICSI procedure.

**Keywords:** ICSI, Sperm Selection, Chromatin, Sperm Functional Test

### **O-15: Motile Sperm Organelle Morphology Examination Is Stricter than Tygerberg Criteria**

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**Background:** The present study aimed to evaluate the correlation between the motile sperm organelle morphology examination (MSOME) and a well-known

sperm morphology classification (Tygerberg criteria).

**Materials and Methods:** Semen samples (one per subject) were obtained from 97 men (mean age 36.7±6.0 years) from an unselected group of couples undergoing infertility investigation and treatment. A portion of each semen sample was processed for MSOME and the remainder of the same sample was analysed by Tygerberg's. For MSOME, the morphological evaluation was performed after semen capacitation. Spermatozoa were analysed at x8400 magnification by inverted microscope (Eclipse TE 2000U Nikon, Japan) equipped with Nomarski differential interference contrast optics, Uplan Apo x100 oil/1.35 Background lens and variable zoom lens (C-mount). A sperm cell exhibiting a normal nucleus (normal shape and chromatin content), as well as a normal acrosome, postacrosomal lamina, neck, tail and mitochondria, and no cytoplasm around the head was classified as morphologically normal. At least 200 spermatozoa were evaluated and the percentage of normal spermatozoa was determined. By Tygerberg's strict criteria the fresh semen underwent morphological evaluation as described in the literature. For spermatozoa to be considered normal, the sperm head, neck, midpiece and tail must be normal. One observer, blinded to subject identity, performed the entire study (MSOME and Tygerberg's). Normal form percentages by Tygerberg's and by MSOME were treated as a continuous variable for analysis. The Wilcoxon matched-pairs signed-ranks test and the Spearman rank correlation test were used where appropriate. The significance level was set at  $p < 0.05$ .

**Results:** Regression analysis demonstrated significant positive correlation between percentage of normal sperm forms by Tygerberg criteria and by MSOME ( $r = 0.83$ ,  $p < 0.0001$ ). However, the incidence of normal spermatozoa by Tygerberg criteria (9.4%) was significantly higher ( $p < 0.0001$ ) than under MSOME (3.3%).

**Conclusion:** Despite the highly positive correlation, MSOME is a much stricter criterion of sperm morphology classification, since it identifies vacuoles and chromatin abnormalities that are not evaluated with the same precision by the analysis of Tygerberg criteria. MSOME should be included among the routine criteria for semen analysis. In addition, MSOME should be used for selection of spermatozoa for intracytoplasmic sperm injection based on the already published literature, as this is a good selection tool.

**Keywords:** MSOME, Semen Analysis, Sperm Nuclear Vacuoles, Tygerberg Criteria

## O-16: Human Dietary Patterns and Semen Quality

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**Background:** Are dietary patterns, substantiated by biomarkers, associated with semen quality?

**Materials and Methods:** In 161 men of subfertile couples undergoing *in vitro* fertilization treatment in a tertiary referral clinic in Rotterdam, the Netherlands, we assessed nutrient intakes and performed principal component factor analysis to identify dietary patterns. Total homocysteine (tHcy), folate, vitamin B12 and B6 were measured in blood and seminal plasma. Semen quality was assessed by sperm volume, concentration, motility, morphology and DNA fragmentation index (DFI). Linear regression models analyzed associations between dietary patterns, biomarkers and sperm parameters, adjusted for age, body mass index (BMI), smoking, vitamins and varicocele.

**Results:** The 'Health Conscious' dietary pattern shows high intakes of fruits, vegetables, fish and whole grains. The 'Traditional Dutch' dietary pattern is characterized by high intakes of meat, potatoes and whole grains and low intakes of beverages and sweets. The 'Health Conscious' diet was inversely correlated with tHcy in blood ( $\beta = -0.07$ ,  $p = 0.02$ ) and seminal plasma ( $\beta = -1.34$ ,  $p = 0.02$ ) and positively with vitamin B6 in blood ( $\beta = 0.217$ ,  $p = 0.01$ ). An inverse association was demonstrated between the 'Health Conscious' diet and DFI ( $\beta = -2.81$ ,  $p = 0.05$ ). The 'Traditional Dutch' diet was positively correlated with red blood cell folate ( $\beta = 0.06$ ,  $p = 0.04$ ) and sperm concentration ( $\beta = 13.25$ ,  $p = 0.01$ ).

**Conclusion:** The 'Health Conscious' and 'Traditional Dutch' dietary patterns seem to be associated with semen quality in men of subfertile couples

**Keywords:** Nutrition, Assisted Reproduction, Environmental Effects, Infertility, Sperm Quality

## Epidemiology and Ethics

### O-17: Survey the Psychological Disorder of Infertility in Infertile Couples (Couples who Undergoing for ART Protocol)

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**Background:** Survey the Psychological disorder of infertility in infertile couples (couples who undergoing for ART protocol): Parenthood is one of the major transitions in adult life for both men and women, which is associated with major emotional sequels. This study was performed to determine the psychological impact of infertility in infertile female (IF) and their partners- normal healthy male (NHM) compared to infertile male (IM) and their partners -normal healthy female (NHF).

**Materials and Methods:** In this descriptive analytical study, 256 subjects who attended for IVF/ICSI treatment program recruited. 78 (IM) and their (NHF) partners; 50 (IF) and their (NHM) partners agreed to completed the symptoms checklist SCL-90 and Cattle anxiety questionnaires. The Persian version of checklist SCL-90 and Cattle anxiety questionnaires were used to evaluate psychological problems such as anxiety, depression, hypochondriacs, obsession, compulsion, aggression, paranoia, phobia, and psychotics in relation to patients by cause of infertility, duration of infertility, age, education, and employment of the patients and their partners.

**Results:** This study showed that, the level of anxiety, depression, hypochondriacs, paranoia, obsession, compulsion, aggression, paranoia, and psychotics were higher than normal average in the all four groups. However, phobia, in (IF) group was higher than (NHF) group. Finding also indicated that (IM) group has higher score on anxiety, depression, compulsion, paranoia, and psychotics in compared (NHM) group. In the other hand, multivariate analysis of variance showed that in (NHM) group, the level of depression, hypochondriacs, paranoia, compulsion and psychotics were lower than the other three groups ( $p < 0.01$ ). In addition, Multivariate regression indicated that in (NHM) group, the level of depression, hypochondriacs, and phobia could predict their anxiety ( $p < 0.01$ ). Overall, Depression in (IM) group, paranoia in (NHF) group and psychotics in (IF) group could be significant predictor of anxiety and depression ( $p < 0.01$ ). The age was another significant predictor for anxiety, aggression (in IF group), obsession, hypochondriacs and psychotics (in NHF group) ( $p < 0.01$ ). Duration of the infertility In (NHF) group was related to lower level of anxiety and depression ( $p < 0.01$ ).

**Conclusion:** It seems that the psychological disorders in infertile women compared to infertile men and their spouse is much higher. And psychological consequences of infertility for women are most. Adequate attention to infertile patients and their partners' psychological aspects and their treatment with cognitive, behavioral therapies and social supports especially for infertile women before IVF/ICSI program is of great importance which may help to improve the quality of life in this population

**Keywords:** Psychological Problems, Infertile Couple, IVF, Infertility

## O-18: A Qualitative Inquiry into Muslim and Christian Infertile Women's Perception of Infertility

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**Background:** Confronting with fertility problem and thinking about encountering infertility in the rest of life as an issue to struggle with is a catastrophe for majority of infertile women. This shocking news is really devastating and like a disaster, which collapses all ambitions, dreams and aspirations of women desiring a child. This study explored how Muslim and Christian women perceive infertility in a religious and spiritual context.

**Materials and Methods:** The design was a grounded theory study including semi structured in-depth interviews with 30 infertile women affiliated to different denominations of Christianity (Protestantism, Catholicism, Orthodoxy) and Islam (Shiite and Sunni). Data were collected in one Iranian and two UK fertility clinics through theoretical sampling and analyzed using grounded theory.

**Results:** The emerged categories embraced abrupt appraising of infertility, subsequent appraising of infertility and challenging for its acceptance, which were encompassed in the core category of relying on a higher being. Abrupt appraising was presented as disbelief, uncertainty, and questioning which was the primary reaction of the women to fertility problem. Subsequent appraising was associated with more searching and probing to find out the cause of the illness and reappraising the meaning of infertility spiritually. In this stage, the majority of religious infertile women presented a positive image of God and viewed infertility as epitome of God's will or God's gift. As a result, they acknowledged their new identity as infertile and tried to cope with the situation adopting religious coping strategies. Their trust and reliance on God and their benevolent reappraisal helped them to be optimistic, hopeful and confident, as they believed in God's wisdom, beneficence and power.

**Conclusion:** Religious participants using a religious/spiritual meaning-making framework tried to reappraise their illness spiritually and trusting a higher power, who can protect individuals, went through different stages and perceived infertility peacefully and as a God-given phenomenon.

**Keywords:** Infertility, Religion, Spirituality, Perception, Grounded Theory

## O-19: Socio-Cultural Beliefs Affecting Infertile Couples' Consideration of Adoption in Iranian Society

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**Background:** Adoption is an alternative strategy in the management of infertility aimed at bringing relief to the affected couples. Although recent advances in reproductive technologies are helping more couples than ever before to achieve their procreative goals, for an estimated 30% to 40% of infertile couples, adoption is their only option for becoming parents. The process of adoption differs between countries depending on the socio-cultural settings and the constitution. This study was designed to investigate the socio-cultural beliefs affecting infertile couples' consideration of adoption in an Iranian population.

**Materials and Methods:** In this analytical survey, 50 infertile couples without and 50 with an adopted-child were selected from urban health clinics in Kashan using stratified sampling. All infertile couples were interviewed in order to take their medical history. Couples' socioeconomic and demographic data were also obtained using a self-administered questionnaire. Subjects' socio-cultural beliefs towards adoption were measured using a self-structured, valid and reliable Likert scale with four subscales including adoption recognition, social perspectives towards adoption, adoptee's identity disclosure and relationship of adoptive parents with birth parents.

**Results:** Two groups had no significant difference in terms of medical history. The total and subscale scores of socio-cultural beliefs were significantly different in couples with and without adopted child ( $p < 0.0001$ ). The highest score of 'adoption recognition' was seen in women with adopted child and the lowest in men without adopted child ( $p < 0.0001$ ). A significant difference was seen between women with adopted child and couples without adopted child ( $p < 0.0001$ ) in relation to 'social perspectives towards adoption' subscale score. The highest score related to 'adoptee's identity disclosure' was observed in women with adopted child and the lowest in men without adopted child ( $p < 0.012$ ). Concerning 'relationship of adoptive parents with birth parents', women with adopted child gained the highest and those without adopted child obtained the lowest score ( $p < 0.0004$ ).

**Conclusion:** Infertile men without adopted-child had more negative views towards adoption recognition, its social implications and disclosure issues, which is probably due to societal beliefs regarding adoption. It is therefore recommended to establish supporting organizations to counsel socio-cultural issues of adoption with infertile couples and to correct the misconception and myths surrounding adoption.

**Keywords:** Infertility, Adoption, Socio-Cultural Beliefs

## O-20: Occurrence of Preterm Deliveries and

## Related Factors in 1513 Pregnancies

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**Background:** Considering the important impact of preterm deliveries on neonatal mortality and morbidity, this study was designed to assess the prevalence of preterm deliveries and evaluate possible effective factors of them, in two cities of Iran.

**Materials and Methods:** In this cross-sectional study on 1513 pregnant women attending delivery facilities in educational hospitals of Tehran and Noor City (in north of Iran), the prevalence of preterm deliveries- which were defined as deliveries before 37th week of gestation- were assessed. After obtaining the consent of subjects to enter the study, a complete pregnancy history was obtained from each subject and related reproductive data along with their demographic characteristics were registered, using a general questioner. In performed analysis,  $p$ -values  $< .05$  were considered significant.

**Results:** Of the 1513 deliveries studied, 103(6.8%) were preterm ( $< 37$  weeks of gestation). We assessed the effect of possible risk factors of preterm deliveries in Binary Logistic Regression Analysis. These factors included: age of mother, previous preterm delivery, number of total pregnancies, active or passive smoking during pregnancy, WBC count, premature rupture of membranes, preeclampsia, consumption of ferrous sulfate, folic acid, calcium and multivitamins during pregnancy, and consumption of folic acid from 3 months before pregnancy. Of all the factors evaluated, only previous preterm delivery (OR:3.89, 95%CI: 1.65-9.19), preeclampsia in pregnancy (OR:1.42, 95%CI: 1.05-1.91), premature rupture of membranes (OR:5.65, 95%CI: 3.28-9.71), and folic acid consumption from 3 months before pregnancy (OR:1.64, 95%CI: 1.02-2.64), had statistically significant effects on preterm delivery.

**Conclusion:** The prevalence of preterm deliveries in this study (6.8%) was close to that of obtained from other studies in Iran (about 7%). All the effective factors in preterm deliveries in this study were expected to be so, except for folic acid consumption from 3 months before pregnancy, which could surprisingly be a risk factor for preterm deliveries. It is recommended to perform more studies in different parts of Iran to confirm the true risk factors of preterm deliveries.

**Keywords:** Pregnancy, Preterm Delivery, Risk Factor

## O-21: Convictions of Health and Well-Being: Islam Women Living with Infertility in India

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**Background:** The Backgrounds of this study are aimed to find out the main relevant risk factors of female infertility and to meet the service needs of people who have problem associated with infertility. To reduce their feelings of social isolation on improved relationship with family and male partners. To promote physical, spiritual and mental health also knowledge and awareness of infertility among focus group.

**Materials and Methods:** This research is initiated for coping, health related quality of life and psychological well-being of women facing the problem of infertility. The research was organized between January to April, 2009 Nellore district rural area in southern part of India, with women facing infertility problems in their family setting. 40 Islam women ages spanned from 20-38 years (with an average of 26.8 years) who do not wish for mother hood via other routes expressed their concern to participate in this research. The period of nonconceiving ranges from 2 - 10 years after marriage (with an average of 8.2 years). The subjects participated in this study expressed infertility is a problem of their social, psychological and stressful to marital life. Subjects income categories are below and just above the national average income. 21% of the participants have secondary education, 46% pre secondary education and the rest 33% are observant religious education. In a gesture to promote health perceptives of the focus group physical exercise, Meditation and counseling protocols were administered for 4 months. Pre and post test scores of cardio-vascular efficiency compared for physical health, Meditation for spiritual health, well-being, anxiety and stress disorders. Causes of infertility, treatment priorities, knowledge and awareness through counseling.

**Results:** The over whelming success in an evidence through paired 't' test results indicated statistically significant increase in cardio-vascular efficiency, spiritual health, well-being, anxiety and stress perceptive and positive marital relationship in infertile islam marginalized female groups. Knowledge, awareness, causes and treatment priorities on infertility also found to be increased among the target group,

**Conclusion:** The findings supported that positive treatment protocols causes significantly infertile females physical and psychological well-being perspectives. This findings lend support to the findings of Hoi-Yanchan, celia et.al(2005) and Geok-ling Lee et.al(2006) infertile people expressed psychological well-being, positive marital relationship and social support through the implementation of coping strategies.

### **O-22: Effectiveness of HIV Counseling Services on Knowledge, Attitude, Behavior and Practice (KABP) among Pregnant Women Attending PPTC Program**

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**Background:** The Background of this study was to assess (KABP) regarding HIV/AIDS among pregnant women attending PPTCT program before and after counseling at Lok Nayak Hospital, New Delhi.

**Materials and Methods:** It was an intervention longitudinal study. Data was collected by interviewing 600 pregnant women attending antenatal clinic during May 2006 to May 2007 using a pre-test and post-tested questionnaire. They were selected based on the eligibility criteria of gestational age between 5-32 weeks. Pregnant women were excluded if they were HIV positive or had received anti-retroviral therapy before attending the clinic. Women who had formal counseling about HIV/AIDS earlier were also excluded. Data was entered and analyzed by using SPSS 16.

**Results:** About 69.2% of the pregnant women had heard about AIDS before the counseling. Knowledge regarding mother to child transmission of HIV was 53.5%. 38.2% knew that mother to child transmission can be reduced by drugs. The knowledge of pregnant women about AIDS was significantly different in pre-test (mean score =15.3) and post test (mean score=35.6) ( $p < 0.0001$ ). Attitude of study participants towards people living with HIV/AIDS (PLWHA) indicated that individuals with HIV should be allowed to work (79.9%) and all commercial sex workers should compulsorily be tested for HIV (94.3%). There was significant difference between in pre-test and post-test attitude about PLWHA and HIV testing ( $p < 0.0001$ ). Overall, condom usage among spouses of study participants significantly improved after counseling [241(47.3%) before and 345 (67.6%) after counseling] ( $p < 0.05$ ).

**Conclusion:** Counseling services were effective in increasing knowledge and changes in attitude and behavior among pregnant women and the efforts needs to be sustained.

**Keywords:** HIV Counseling, Knowledge Attitude Behavior and Practice, Pregnant Women

### **O-23: Conflict and Crisis Setting. Promoting Health and Sexual and Reproductive Health of Women Agriculture Workers in India**

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**Background:** Women in working life have received considerable attention in last few years, this appears to influence the health, sexual and reproductive health (SRH) of women as manifested both in the work place and their family life. Global studies demonstrated that one of the major issues confronting women is health and SRH. The most important Priorities women at work place should be promotion of health, SRH and mental health (Berg and Thereon 2004). Rural women in this study site stated that their access to health and SRH knowledge and services are limited. A proactive policy is required to enhance Institutional mechanism to deliver health and SRH services. Backgrounds: The main Backgrounds of this program is-it is essential that we enlighten and create widespread awareness of education, health and SRH issues among all sections of society, particularly in rural areas where deprived people living and access to this services are very limited.

**Materials and Methods:** Drawing on a study centered health reforms of rural women agriculture workers, NGO carried-out a research project with six policies implementation mechanism. 1. integrating health SRH services. 2. women friendly and client-centered information, education and communication. 3. interventions to improve client-provider communications in health facilities. 4. training for communication about sexual health. 5. capacity building of multi purpose workers in RH. 6. Yoga, relaxation and breathing. The recognized parameters are measured to find out the progress through policy implementations.

**Results:** The project findings demonstrated that a significant progress in health parameter of the subjects (9.59,  $p < 0.05$ ). The six policies implemented mechanism has a very positive outcome in SRH perceptions of the target population.

**Conclusion:** Discussion on the findings: These findings correlated with (Lagerlof 2003) findings on women work and health focusing knowledge on work related health and SRH through suitable interventions.

### **O-24: Prevalence of Primary Infertility in the Islamic Republic of Iran in 2004-2005**

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**Background:** This study aimed to determine the prevalence of lifetime and current primary infertility in Iran. The study was conducted in 2004-5 in all the 28 provinces of Iran.

**Materials and Methods:** The study was conducted in 2004-5 in all the 28 provinces of Iran. We used a cluster sampling method to select 10,783 women aged 19-49 for the survey. Life-time primary infertility was defined

based on one of two contraception scenarios immediately after marriage to find experience of infertility despite one year of unprotected intercourse. The term "current-primary infertility" designated a woman who, in addition to meeting the definition of life-time infertility, had been unable to conceive up to the study time. We used a complex sampling design and SVY commands in the software package STATA 8.0 to derive 95% confidence intervals.

**Results:** A history of life-time primary infertility was present in 24.9% of the subjects (95% CI: 23.5-26.2%), and the prevalence of current-primary infertility was 3.4% (95% CI: 3.0-3.8%). As for age trends in life-time primary infertility, the highest prevalence rates were observed in individuals with the lowest age at marriage. Minimum prevalence (17.2%) occurred with marriage age of 21-26, and the rates rose with higher age at marriage.

**Conclusion:** About one fourth of the Iranian couples experience primary infertility at some point in their lives and 3.4% suffer from this problem at any time. For a correct interpretation of prevalence rates and the implications in terms of health care and service delivery, factors such as age at marriage and the couple's fertility potential must be taken into consideration.

**Keywords:** Current Primary Infertility, Infertility, Iran, Life, Time Primary Infertility, Primary infertility

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## **Female Infertility**

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### **O-25: Single and Dual Embryo Transfer, How Far We Are?**

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**Background:** To explore and compare the dual embryo transfer DET with single embryo transfer SET weather electively or not, in more viewpoints like financially, and scientifically and finding out which of these policies may give better outcome by analyzing both in a neutral broad spectrum manner.

**Materials and Methods:** A computerized comprehensive search studies ended in finding of 62 articles of which 49 are from specialized web sites consisting of randomized and non randomized clinical trials, reviews, comments, analysis, debates, and reference lists was done in the PubMed and the Cochrane Central Register of Controlled Trials (Central).

**Results:** The comparison between all these articles found to be unreliable due to the huge variation between them all, but mainly SET has the advantage of minimizing the twinning and multiple pregnancy rate MPR, on the other hand it has less benefit when compared to DET in the matter of implantation rate IR, ongoing pregnancy

rate OPR, but a comparable results may be obtained when applying eSET of frozen-thawed embryo.

**Conclusion:** Larger clinical trials should still be encouraged for such comparison especially in applying same criteria for both methods.

**Keywords:** Assisted Reproductive Technology, Twins, Single Embryo Transfer, Double Embryo Transfer

### **O-26: Evaluation of the Relationship between Follicular Fluid Anti- Mullerian Hormone Levels and Fertilization and Embryo Quality in ART Cycles**

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**Background:** To evaluate whether success of fertilization and embryo quality are more probable when follicular fluid Anti-mullerian hormone levels are higher .

**Materials and Methods:** 62 women who underwent IVF cycle were divided into fertilized oocytes group (n=42) and non- fertilized oocytes group (n=20) . Follicular fluid Anti-mullerian hormone levels were measured in both groups.

**Results:** Anti-mullerian hormone levels in fertilized group were higher than non-fertilized group and a statistically significant difference was observed between the groups. Anti-mullerian hormone levels and high quality embryos were found to be correlated and the difference was statistically significant .

**Conclusion:** Our results indicate that follicular fluid Anti-mullerian hormone levels have positive relationship with fertilization and embryo quality , so we can use follicular fluid Anti-mullerian hormone levels as an indicator of IVF outcome.

**Keywords:** Anti-Mullerian Hormone, Follicular Fluid, Fertilization, Embryo Quality, *In vitro* Fertilization

### **O-27: Treatment of Ovarian Hyperstimulation Syndrome Using Gonadotropin Releasing Hormone Antagonist**

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**Background:** Ovarian hyperstimulation syndrome is the most serious complication of ovarian stimulation. This novel study aimed to describe an outpatient treatment protocol for ovarian hyperstimulation syndrome (OHSS) that results in rapid normalization of symptoms with minimal side effects.

**Materials and Methods:** A prospective study was undertaken at Infertility department of Shariati Hospital af-

filiated to Tehran University of Medical Sciences during 2009-2010. Ten consecutive patients undergoing controlled ovarian hyperstimulation after long protocol pituitary down regulation, diagnosed with OHSS, who presented after oocyte retrieval but without embryo transfer, were enrolled. All embryos were frozen and each patient was treated with the daily 0.25 mg(sc) Cetrotide for two consecutive days. Weight changes, days to resolution of clinical symptoms, side effects of the treatment protocol, and whether or not acute care or hospitalization was necessary were evaluated. They were compared with other ten OHSS patients (control group) managing with conservative measures and dopamine agonist (cabergoline).

**Results:** Interesting results of this study revealed that the average time to resolution of clinical symptoms with Cetrotide was shorter. No side effects were reported and no patients required acute care or hospitalization. The most rapid weight loss was within the first week of treatment. The Patents' satisfaction with Cetrotide was noticeable.

**Conclusion:** GnRH antagonists, when given at the time of diagnosis of OHSS, appear to work rapidly and effectively to diminish the clinical symptoms of the disease. May be it is attributed to its luteolytic effect. Due to debate about safety of these drugs during pregnancy it is reasonable to freeze the embryos for future cycles.

**Keywords:** OHSS, GnRH Antagonist, Treatment

### **O-28: New Insights into the Mechanisms Underlying Chlamydia Trachomatis Infection Induced Female Infertility**

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**Background:** Chlamydia (C.) trachomatis is an obligate intracellular gram-negative pathogen affecting over 600 million people worldwide with 92 million new cases occurring globally each year. Genital C. trachomatis infection has been recognized as the most common cause of pelvic inflammatory disease leading to severe tubal damage, ectopic pregnancy, hydrosalpinx and infertility. However, the mechanism underlying hydrosalpinx induced by C. trachomatis infection remains largely unknown.

**Materials and Methods:** Real time – polymerase chain reaction, western blot analysis and immunoprecipitation, histology including Masson's trichrome staining, immunostaining, electron microscopy, Chlamydia infection rodent models and Cystic fibrosis transmembrane

conductance regulator (CFTR) mutant mice were used in this project. Sperm motility and acrosome reaction were determined using computer-aided sperm analysis (CASA) and acrobead assay respectively, and embryo development by mouse embryo development assay.

**Results:** We first characterized hydrosalpinx of infertile patients seen on ultrasound scan. Inflammatory cells could be found in hydrosalpinx fluid (HF) in the lumen in areas with flattened to no epithelial cells, as well as dilated blood vessels and/or lymph vessels. Scanning electron microscopy revealed severe loss of both cilia and microvilli and for the first time stomatae exuding globular bodies on eroded ampullae surfaces providing explanation for HF formation, and thus for the detrimental effects of HF on reproductive processes and IVF outcome. Further investigation using Masson's trichrome staining showed areas of epithelial transformation, focally attenuated and pseudostratified epithelium. Immunostaining showed enhanced CFTR immunoreactivity in the areas of transformed hydrosalpinx epithelium. Correlation with *C. trachomatis* infection was done by testing hydrosalpinx patients' sera for *C. trachomatis* immunoglobulin G antibody titers using a Capita enzyme-linked immunosorbent assay (ELISA) based kit. We then determined CFTR involvement using a rodent *C. trachomatis* infection model and confirmed it using CFTR mutant (CFTR (tm1Unc)) mice. Increased CFTR expression and fluid accumulation was observed in the uterine horns infected with *C. trachomatis* elementary bodies. We further showed that upregulated CFTR expression and consequent fluid accumulation led to decreased implantation and infertility using a mouse model. For *C. trachomatis* to cause infection, it has to enter epithelial cells. However, the exact mechanism or receptor(s) for *C. trachomatis* reproductive tract epithelial entry is not well understood. Using human epithelial cell lines expressing functional and mutant Delta508 CFTR cells, CFTR mutant mice and wild type controls, we for the first time demonstrate that CFTR functions as a cell surface receptor for epithelial cell entry and internalization of *C. trachomatis*.

**Conclusion:** The findings of this project may lead to the development of new treatment strategies to curtail the spread of Chlamydial infections, reduce hydrosalpinx formation and improve assisted reproduction treatment outcome in infertile patients.

**Keywords:** Female Infertility, Chlamydia Trachomatis Infection, Gene Expression, Pathogenesis, Hydrosalpinx, IVF Outcome

### **O-29: Pregnancies Following the Use of Sequential Treatment of Metformin and Incremental Doses of Letrozole in Clomiphene-Resistant Women with Polycystic Ovary Syndrome**

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**Background:** Clomiphene citrate (CC) is the first line therapy for women with infertility and polycystic ovary syndrome (PCOS). However, 20-25% of women are resistant to CC and do not ovulate. The Background of this study is to evaluate the efficacy of sequential treatment of metformin and incremental doses of letrozole in induction of ovulation in cases of clomiphene citrate-resistant (CC-R) PCOS patients and to determine in which doses of letrozole the maximum response would occur.

**Materials and Methods:** In this prospective trial, we enrolled 106 anovulatory PCOS women who failed to ovulate with CC alone from Amir-almomenin University General Hospital in Semnan, Iran. After an initial 6-8 weeks of metformin treatment, they received 2.5mg letrozole for 5 days starting from day 3 of their menstrual cycle. If they did not ovulate with 2.5mg letrozole, the doses were increased to 5 to 7.5 mg daily in subsequent cycles. The main outcomes were ovulatory and pregnancy rates in different doses of letrozole and cumulative pregnancy rate at the end of treatment.

**Results:** 14 of 105 patients (13.33%) conceived with metformin alone. The remaining 91 patients who did not conceive with metformin continued metformin and letrozole was started for them. Ovulation occurred in 83 out of 91 patients (91.2%) and maximum ovulation occurred in 2.5mg (40.65%) and 5mg (37.36%) doses of letrozole respectively. Only 13.8% of ovulation occurred in 7.5 mg letrozole. In the end, 8 out of 91 patients (8.79%) remained anovulatory. Cumulative pregnancy rate was 60/105 (57.14%) with 45 out of 60 (74.9%) pregnancies being full term.

**Conclusion:** Combination of metformin with incremental doses of letrozole is associated with a good pregnancy rate in CC-resistant PCOS patients.

**Keywords:** CC-resistant, Induction Ovulation, Letrozole, Metformin, PCOS

### **O-30: Novel Interventions to Reduce Re-infection in Women with Chlamydia: A Randomised Controlled Trial**

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**Background:** To determine if postal testing kits (PTK) and patient delivered partner therapy (PDPT) for managing sexual partners of women with Chlamydia trachomatis

matitis, reduce re-infection rates in women, compared to partner notification by patient referral.

**Materials and Methods:** Three hundred and thirty women testing positive for chlamydia, at clinics for genitourinary medicine, family planning and termination of pregnancy in Edinburgh, were randomized to one of three partner interventions: patient referral, PTK (partners post urine for testing) or PDPT (1 g azithromycin for partners). Women submitted urine for chlamydia testing every 3 months. The primary outcome was re-infection assessed as time to first positive result by the Cox proportional hazard regression. The proportion of partners tested or treated with each intervention was determined.

**Results:** Out of 330 women, 215 (65%) were retested over 12 months. There were 32 of 215 women (15%) who retested positive (7, 15 and 10 women from the patient referral, PTK and PDPT groups, respectively). There was no significant difference in re-infection between PDPT versus patient referral (HR 1.32, 95% CI 0.50 – 3.56), PTK versus patient referral (HR 2.35, 95% CI 0.94 – 5.88) or PDPT versus PTK (HR 0.55, 95% CI 0.24 – 1.24). There was no significant difference in the proportion of partners confirmed tested/treated between the patient referral (34%) and PTK (41%,  $p=0.32$ ) or PDPT (42%,  $p=0.28$ ) groups.

**Conclusion:** PTK and PDPT do not reduce re-infection rates in women with chlamydia compared with patient referral. However, PDPT may offer other advantages such as simplicity and cost compared with patient referral.

**Keywords:** Chlamydia Trachomatis, Patient-Delivered Partner Therapy, Partner Notification, Postal Testing Kits, Re-Infection

### **O-31: Mifepristone Acts as Progesterone Antagonist of Non-Genomic Responses but Inhibits Phytohemagglutinin Induced Proliferation in Human T Cells**

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**Background:** Progesterone is an endogenous immunomodulator that suppresses T cell activation during pregnancy. The stimulation of membrane progesterone receptors (mPRs) would seem to be the cause of rapid non-genomic responses in human peripheral T cells, such as an elevation of intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) and decreased intracellular pH (pHi). Mifepristone

(RU486) produces mixed agonist/antagonist effects on immune cells compared with progesterone. We explored whether RU486 is an antagonist to mPRs and can block rapid nongenomic responses and the induction by phytohemagglutinin (PHA) of cell proliferation.

**Materials and Methods:** Human male peripheral T cell responses in terms of pHi and [Ca<sup>2+</sup>]<sub>i</sub> changes were measured using the fluorescent dyes, 20,70-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) and fura-2, respectively. Expression of mPR mRNA was determined by RT-PCR analysis. Cell proliferation and cell toxicity were determined by [<sup>3</sup>H]-thymidine incorporation and MTT assay, respectively.

**Results:** The mRNAs of mPRa, mPRb and mPRg were expressed in T cells. RU486 blocked progesterone-mediated rapid responses including, the [Ca<sup>2+</sup>]<sub>i</sub> increase and pHi decrease, in a dose related manner. RU486 did not block, but enhanced, the inhibitory effect of progesterone on PHA induced cell proliferation. RU486 alone inhibited proliferation induced by PHA and at >25 uM seems to be cytotoxic against resting T cells ( $p<0.01$ ).

**Conclusion:** RU486 is antagonistic to the rapid mPR-mediated non-genomic responses, but is synergistic with progesterone with respect to the inhibition of PHA-induced cell proliferation. Our findings shine new light on RU486's clinical application and how this relates to the non-genomic rapid physiological responses caused by progesterone.

**Keywords:** Progesterone, Mifepristone, RU486, Membrane Progesterone Receptors, T Cells

### **O-32: Status of Human ART in Spain: Results from the New Registry of Catalonia**

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**Background:** FIVCAT.NET is the registry of human assisted reproductive techniques (ART) in Catalunya to which all authorised centres are obliged to declare their activities. The Background of the present study is to describe the data on effectiveness of the ART in Catalunya over the period 2001-2005 and to compare our findings with other similar registries.

**Materials and Methods:** The data were obtained from the centres conducting ART over this period. All users of the services were included, whether resident or non-resident in Catalunya. Descriptive data of the different stages of the ART process (oocyte recovery, embryo transfer, clinical pregnancy and births) were analysed. The effectiveness of ART was measured by indicators such as rates of pregnancy and rate of live birth per pregnancy.

**Results:** An increase (92.4 %) in ART activity over this period was noted. The number of embryos most fre-

quently transferred changed from three to two. Effectiveness indicators such as pregnancies per transfer improved from 33.2 to 37.1 (from 36.9 to 40.2 using fresh embryos and from 18.4 to 27.0 with frozen embryos). Multiple births decreased from 50.1 to 38.6, premature births from 37.5 to 28.3 and low-birth-weight infants from 38.0 to 25.6.; findings which compare favourably with other countries .

**Conclusion:** The data collected by FIVCAT.NET are comparable to other surrounding countries, not only with respect to level of activity but also to outcomes achieved.

**Keywords:** Assisted Reproductive Techniques, Fertility, Births, Epidemiologic Study

### **O-33: Can Unilateral Ovariectomy Be Used as A Method of Infertility Treatment in Case of Resistant Polycystic Ovary Syndrome (Experimental Research)**

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**Background:** Polycystic ovary syndrome (PCOS) is an endocrine disturbance that causes primary anovulation that result in infertility. There are more symptoms accompanying this syndrome: acne, hirsutism, obesity, irregular menstrual cycle and many others. This syndrome can be more or less successfully treated but in 10-15% of cases it is resistant to any kind of treatment used in nowadays practice. The aim of our research was to give a background to the usage of unilateral ovariectomy in reducing the severity of PCOS in case of resistant forms of this syndrome.

**Materials and Methods:** Experiment was carried out on 72 Wistar female rats. 18 rats served as a control. We performed unilateral ovariectomy (UOE) on all of the rats from experimental group. Terms of observation were determined in 1, 3 and 6 month after the surgical intervention in order to compare the changes taking place in the ovary that was left.

**Results:** It was revealed that in normal ovaries in 6 month after UOE the amount of growing and mature follicles is considerably decreased in comparison with the control group. In case of polycystic changed ovaries in 3 month after UOE it was revealed that partial restoration of the ovogenesis takes place. In 6 month after UOE performed on the ovaries with PCOS the formation of the yellow bodies was noted, which is not characteristic for this syndrome and tells about ovulation that took place in the ovary.

**Conclusion:** Unilateral ovariectomy can be advised as the method of treatment of the resistant forms of polycystic ovary syndrome.

**Keywords:** Polycystic Ovary Syndrome, Unilateral Ovariectomy, Infertility

### **O-34: Improvement of Pregnancy Rate in ART Cycles**

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**Background:** The aim of this study was to determine the outcome of IVF/ ICSI by modification of embryo transfer technique following embryo transfer.

**Materials and Methods:** In this randomized trial study, two groups of infertile women (n = 55) aged %40 years underwent *in vitro* fertilization or intracytoplasmic sperm injection treatment cycles with or without 0.2 mL of air pushed into the catheter after embryo transfer

**Results:** The implantation and clinical pregnancy rates were statistically significantly higher in the study group than in the controls.

**Conclusion:** This improvement on standard ET technique may advance clinical pregnancy rates.

**Keywords:** Embryo Transfer Technique, IVF, ICSI, Pregnancy Rate

### **O-35: How Can You Decrease the Complication of Ovarian Diathermy in Polycystic Ovarian Syndrome's Patients?**

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**Background:** PCOS is the most common endocrinopathic and reproductive disorders in women. The pathogenesis is still controversial. There has long been an abnormal gonadotropin secretion with this syndrome.

Hyperandrogenemia is principally ovarian in origin although the adrenal gland may contribute. pcos is associated with relevant reproductive morbidity including, menstrual irregularity, anovulation, infertility, increased pregnancy loss, and complication of pregnancy loss. The aim of this research, decrease the complication of ovarian diathermy in polycystic ovarian syndrome's patients.

**Methods and Materials:** This prospective clinical research evaluates the influential of ovarian diathermic laparoscopic factor in pcos patients that unresponsive to medical therapy. During 4 years, 177 patients with pcos were operated due to unresponsive to medical therapy and IUI , therefore, laparoscopic bilateral monopolar ovarian cauterization in 9 to 15 points at 70 watt cutting power and a 40 watt coagulating power setting and as the probe is pushed into the capsule, electricity

is activated for 4-5 seconds were performed. It is necessary to avoid damage of the ovarian hilum. The probe is applied to the surface of the ovary at a right angle, to avoid slippage and to minimize surface damage, with the depth of penetration 4-5mm. At the end of ovarian drilling was cooled down by irrigation using saline normal solution(500 - 1000cc).

**Results:** No accessible 44 patients, but in following up 133 pcos ladies, 86 conception(64.6 %), 58.7% term pregnancy, 5.9% miscarriage. FSH level, were tested in the rest of 47 patients that was reported normal, after following up for 6 years after the first laparoscopy. So, there aren't any evidence of ovarian failure in all patients.

**Conclusion:** The application of optimal technique is suggested for laparoscopic ovarian surgery and the numbers of punctures in per ovary must be depended to ovarian volume. Despite, duration, depth of diathermy and intensity of power setting are important. View of situation can be prevented ovarian failure, so fertility preservation will be ensured.

**Key words:** PCOS, Ovarian Cauterization, Irrigation, Ovarian Volume

### **O-36: The Role of Galectin-3 in Rat Corpus Luteum Maintenance**

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**Background:** Galectin, an animal lectin that recognizes b-galactosides of glycoconjugates, is involved in multiple biological functions such as cell growth, differentiation, apoptosis, signal transduction and angiogenesis. Overexpression of galectin-3 was observed in different tumors which was related to pro-angiogenic activity. In addition, galectin-3 is intensely expressed in corpus luteum (CL) which was proposed to be associated with CL remodeling but the exact mechanism by which it works is not yet clear. Since angiogenesis play an important role in CL remaining or demise, here we sought to determine the role of galectin-3 in CL angiogenesis by using modified citrus pectin (MCP/Pectasol) as a known component to inhibit pro-angiogenic properties of galectine-3.

**Materials and Methods:** Immature female Wistar rats weighting between 40-50g were stimulated with intraperitoneal injection of PMSG (10IU) (n=4). After 48h, anesthesia was performed three hours prior to intraperitoneal hCG injection and ovarian intrabursal injection of one ovary per animal was performed with a total volume of 50 ul containing 0.5 mg/ml MCP dissolved in phosphate buffer solution (PBS). Contralateral ovary was injected with equal volume of PBS and used as control. Ovaries were removed 48 hours post hCG stimulation, paraffin embedded and sectioned (5 um). The total number of CL (healthy + apoptotic) was counted in MCP-treated and control ovaries every twenty section and results were expressed as Mean  $\pm$  SEM. Endothelial cell

density in CL was assessed by immunohistochemistry analysis using goat monoclonal anti-PECAM/CD31 antibody. The intensity of immunostaining was quantified by using a software using C language (Microsoft Visual Studio 2005 and Dot Net framework 2.0). Statistical analysis was performed by using SPSS version 16.

**Results:** The number of CL in regression was higher in MCP-treated ( $11.0 \pm 0.68$ ) compared to control ( $9.8 \pm 0.62$ ) ovaries, which was not reach significance. We found a significant decreased endothelial cell density in CL of MCP-treated ovary compared to CL of control ovary as revealed by decreased PECAM immunostaining ( $p < 0.001$ ).

**Conclusion:** This is the first evidence for a pro-angiogenic role of galectin-3 in a model of physiological angiogenesis. Thus, this molecule may play an important role in CL maintenance and it would be worth to further investigate its relation with infertility due to progesterone insufficiency.

**Keywords:** Galectin-3, Angiogenesis, Corpus Luteum Maintenance

### **O-37: Effect of Weight Reduction on Ovarian Functions in Anovulatory Overweight Women with Polycystic Ovaries**

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**Background:** The prevalence of obesity is continuing to increase among women. Irregular menstrual cycles, reduced spontaneous and assisted fertility and an increased risk of miscarriage are risks associated with obesity that are often overlooked. This prospective study evaluated the effect of weight reduction on ovarian functions and fertility in anovulatory overweight women with polycystic ovary syndrome (PCOS).

**Materials and Methods:** Forty one anovulatory overweight women (BMI > 24.9 - 30) with PCOS were enrolled in the study. All had chronic anovulation and first or second infertility. Thirty four of them were oligo-amenorrhoeic. Their partners were normospermic. they had not tubal factor or other female factors. For all women diet and exercise was recommended. Ovarian imaging and parameters and fertility rate were assessed at baseline and after weight loss of 5 and 10%.

**Results:** Twenty nine patients (70.7%) lost at least 5% of their body weight. nine of these patients (21.9%) reached a 10% decrease in weight. Ovarian morphology changed during the diet (we observed a significant reduction in ovarian volume and in the number of micro-follicles per ovary). Among the 34 patients with oligo-amenorrhoea, 21(61.8%) had a resumption of regular cycles and 14 ( 41.2%) experienced spontaneous ovulation. All spontaneous pregnancies occurred in women

who lost at least 5% of their weight. and 9 women were in group with 10% decrease in weight .

**Conclusion:** Weight loss through a controlled diet improves ovarian function (restore ovulatory cycles) and morphology (volume and microfollicle number). Also can allowing spontaneous pregnancy in overweight PCOS women.

**Keywords:** Polycystic Ovaries, Weight Reduction, Overweight, Anovulation

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## Genetics

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### **O-38: Evaluation the Expression of Bax, Bcl-2, p53 & Survivin after Transplantation of Spermatogonial Stem Cells to Cryptorchid Mouse Model**

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**Background:** Disruption in spermatogenesis and infertility occurred after local testicular heating. In this study in addition to evaluation the long term effects of experimental bilateral and unilateral cryptorchidism on sperm parameters and structure of mouse testis, the genes that involved in programmed cell death after cryptorchidism and treatment methods of decreasing the germ cells in seminiferous tubules after heat exposure were examined.

**Materials and Methods:** To induce bilateral and unilateral cryptorchidism model, immature mice (age under 2 months) were anesthetized, a small incision was made in the abdominal skin and then fat pad at the upper end of testis was sutured to peritoneum. Testes were removed after 2, 4, 6 and 8 weeks after surgery, weighted and processed for light microscopy study. Expression of apoptotic genes (Bax, Bcl-2, p53 and survivin) and their proteins were evaluated at different times after surgery with semiquantitative RT-PCR and immunohistochemistry methods. In next steps, Sertoli and spermatogonial cells were isolated from cryptorchid mice testes using two step enzymatic digestion and lectin immobilization. The identity of the isolated cells was confirmed by analysis of immunocytochemistry against oct-4 and vimentin. Isolated spermatogonial cells were co-cultured with Sertoli cells three weeks. Colony assay was performed by means of a light microscopy at the end of every week. Finally two treatment methods were examined. In transplantation group, cultural spermatogonial cells were marked with BrdU and with 105 concentrations transplanted into seminiferous tubules of left testis of cryptorchid mouse 3 months after descending surgery and then returned to scrotum. In another group, testis of mouse model returned to scrotum 2 months after ascending surgery. Eight weeks after treatment, testicular structure, ultra structure, expression of apoptotic

genes and proteins were examined. Statistical analysis performed by statistical tests using ANOVA and Tukey's methods.

**Results:** Spermatogenesis was arrested and the testicular weight and seminiferous tubular diameters were significantly reduced in the bilateral undescended testis compared with unilateral undescended testis and the control mice. Spermatocytes and spermatids were the main type of germ cells undergoing apoptosis in all groups. RT-PCR data further verified the alteration of p53, Bax and Bcl-2 mRNA expression in cryptorchid testis in a time dependent manner. The expression of survivin 225 and 140 strongly decrease in bilateral groups comparing with unilateral and control groups, but survivin 332 was lower in both experimental group. These changes were remarkable in bilateral group in compare to unilateral group. Immunohistochemistry data showed that the intensity of p53 and Bax expression mainly increased in remainder cells in cryptorchid testis and rates of Bcl-2 and survivin expression mainly in bilateral group decreased. Transplantation of spermatogonial stem cells into the mouse seminiferous tubules was successful in recipients which had severe tubular degeneration after induction of cryptorchidism compared with the group that testis was descend to scrotum. After transplantation of spermatogonial stem cell in to the cryptorchid testis, germ cell colonization was shown, the number of spermatogonia, spermatocytes returned to near normal range but spermatogenesis was recovered partially at the late stages of spermatogenesis.

**Conclusion:** In general, high temperature causes a decrease in all analyzed parameters except spermatogonial cell number in consequently this model is suitable for enrichment of spermatogonial stem cells. RT-PCR and immunohistochemistry methods observations suggest that multiple molecular pathways participate in the germ cell apoptosis induced by cryptorchidism. The reason of partially return of spermatozoa after transplantation maybe is the severe effects of surgery on the blood supply or innervations of the testis and also disruption in reconstruction of serous sac around the testis without adhesions, which are important for normal spermatogenesis.

**Keywords:** Apoptotic Genes, Cryptorchidism, Transplantation, Mouse Testis

### **O-39: Evaluation the Expression of Bax, Bcl-2, p53 & Survivin After Treatment of Cryptorchidism in Mouse**

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**Background:** Experimental cryptorchidism is a common model for examining the expression and function of heat-induced spermatogenesis related genes in testis. In this study the expression of genes & proteins that

involved in programmed cell death after treatment of cryptorchidism was examined.

**Materials and Methods:** To induce bilateral cryptorchid model immature mice were anesthetized and a small incision was made in the abdominal skin and peritoneum then fat pad at the upper end of testis sutured to peritoneum. After performance of two treatment methods, transplantation of germ stem cells and orchidopexy molecular examination and protein expression of Bax, p53, Bcl-2 and Survivin genes were evaluated at 2 & 8 weeks after treatment comparing with control and bilateral groups.

**Results:** RT-PCR data showed the decreased of p53 & Bax expression as well as decreased of Bcl-2 mRNA in treatment groups especially after transplantation compared with control group. The expression of survivin 140 significantly increased after treatment, where as expression of survivin 40 was lower especially in orchidopexy group. Immunohistochemistry staining showed that the intensity of Bax expression mainly decreased in treated cryptorchid testis and rates of Bcl-2 increased significantly but expression of p53 and survivin proteins not many changed after treatment.

**Conclusion:** These observations suggest that multiple molecular pathways participate in the germ cell apoptosis induced by treatment of cryptorchidism.

**Keywords:** Apoptotic Genes, Cryptorchidism, Mouse

#### **O-40: MTHFR Promoter Hypermethylation in Testicular Biopsies of patients with Non-Obstructive Azoospermia: the Role of Epigenetics in Male Infertility**

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**Background:** MTHFR promoter hypermethylation in testicular biopsies of patients with non-obstructive azoospermia: the role of epigenetics in male infertility.

**Materials and Methods:** DNA from peripheral blood (PB) samples of 50 patients with NOA and 50 fertile men (controls) as well as DNA from testicular biopsies of 32 patients with NOA and five patients with obstructive azoospermia, but normal spermatogenesis, were analyzed by Methylation Specific PCR amplification using primers that hybridize to the CpG island in the promoter region of MTHFR.

**Results:** In PB, no differences in the methylation profile of the promoter region of MTHFR were observed between patients and controls. In testis biopsies, hypermethylation was detected in 53% of the patients with

NOA compared with 0% of patients with obstructive azoospermia ( $p = 0.03$ ).

**Conclusion:** These results indicate that hyper-methylation in testis DNA from NOA patients is specific and not due a general methylation defect, and suggest that epigenetic silencing of MTHFR could play a role in azoospermic infertility.

**Keywords:** MTHFR, Azoospermia, Methylation, Epigenetics

#### **O-41: Comparison of Vitrification Systems in Human Oocytes and Evaluation of HSP A1A and MnSOD Gene Expression Patterns**

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**Background:** The aim of this study was to compare two vitrification systems (Cryotop and OPS) on mouse MII oocytes and evaluate its effects on expression of heat shock protein A1A and MnSOD genes.

**Materials and Methods:** Groups of about 15 oocytes without cumulus complexes (granulose, et. al.) were collected and exposed to equilibration and vitrification solutions before loading the oocytes into open pulled straws (OPS method) or Cryotop, and carriers transferred to liquid nitrogen. For thawing the oocytes are exposed to three solutions of gradually declining concentrations of sucrose. After thawing live oocytes were subjected to IVF and cultured *in vitro* to develop into pronuclear stage. Survival of vitrified-warmed oocytes and rate of fertilization were evaluated; gene expression (HSP A1A, MnSOD and  $\beta$ -actin) of vitrified-warmed oocytes were also examined by reverse transcriptase polymerase chain reaction (RT-PCR).

**Results:** Survival rates of each group were separately compared to control and the results showed that there were significant differences between each experimental group as compared to control ( $p \leq 0.05$ ) reduced in vitrified-warmed (Cryotop: 39 %, 34 %, OPS: 29.2 %, 19.7 %) oocytes compared to control (88.36). The expression of MnSOD increased in vitrified-warmed samples as compared to control oocytes. We also detected HSP A1A only in the control (EG + DMSO) group using either Cryotop or OPS.

**Conclusion:** Vitrification of oocytes by cryotop resulted in higher survival rate, lower developmental competence, and lower fertilization rate of vitrified-warmed oocytes may be because of changing expression rates of important genes after thawing. Further genetic screens can be done to determine expression patterns involved in this effect.

**Keywords:** Cryotop, OPS, Gene Expression, Oocyte, Vitrification, HSP A1A, MnSOD

### **O-42: Expansion of CAG Repeats in the Spinocerebellar Ataxia Type 1 (SCA1) Gene in Idiopathic Oligozoospermia Patients**

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**Background:** The lengths of CAG repeats in two spinocerebellar ataxia genes, SCA1 and SCA3, were analyzed to determine whether such repeats exist in higher numbers in infertile males.

**Materials and Methods:** Blood samples were collected from healthy controls, oligozoospermia patients, and azoospermia patients. DNA fragments containing target CAG repeats were amplified by PCR with template DNA purified from the blood samples. CAG repeats in PCR fragments were determined, using ABI PRISM 310 Gene Analyzer.

**Results:** In SCA1, the distribution of CAG repeats in oligozoospermic males was different from that of the control group: More alleles had a repeat number that exceeded 32. Conversely, for SCA3, the examined oligozoospermia and azoospermia patients exhibited no differences in distribution of CAG repeats in comparison with the control group.

**Conclusion:** SCA1 in a subset of oligozoospermia patients has an increased number of CAG repeats.

**Keywords:** CAG Repeats, Oligozoospermia, Spinocerebellar Ataxia Type 1 Gene, Spinocerebellar Ataxia Type 3 Gene

### **O-43: Mutations in NR5A1 Associated with Ovarian Insufficiency**

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**Background:** The genetic causes of nonsyndromic ovarian insufficiency are largely unknown. A nuclear receptor, NR5A1 (also called steroidogenic factor 1), is a key transcriptional regulator of genes involved in the hypothalamic–pituitary–steroidogenic axis. Mutation of NR5A1 causes 46,XY disorders of sex development, with or without adrenal failure, but growing experimental evidence from studies in mice suggests a key role for this factor in ovarian development and function as well.

**Materials and Methods:** To test the hypothesis that mutations in NR5A1 cause disorders of ovarian development and function, we sequenced NR5A1 in four families with histories of both 46,XY disorders of sex development and 46,XX primary ovarian insufficiency and in 25 subjects with sporadic ovarian insufficiency. None of the affected subjects had clinical signs of adrenal insufficiency.

**Results:** Members of each of the four families and 2 of the 25 subjects with isolated ovarian insufficiency carried mutations in the NR5A1 gene. In-frame deletions and frameshift and missense mutations were detected. Functional studies indicated that these mutations substantially impaired NR5A1 transactivational activity. Mutations were associated with a range of ovarian anomalies, including 46,XX gonadal dysgenesis and 46,XX primary ovarian insufficiency. We did not observe these mutations in more than 700 control alleles.

**Conclusion:** NR5A1 mutations are associated with 46,XX primary ovarian insufficiency and 46,XY disorders of sex development.

**Keywords:** NR5A1, Female Infertility, Ovarian Insufficiency, 46XY DSD

### **O-44: Characterisation of Monotreme Caseins Reveals Lineage Specific Expansion of an Ancestral Casein Locus in Mammals**

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**Background:** One important reproductive characteristic of Mammals is the production of milk to nurse the neonate. In order to better understand the evolution of milk we have investigated gene expression in milk cells from monotremes which are the most ancient representative of the mammalian lineage.

**Materials and Methods:** Using a milk cell cDNA sequencing approach we characterise milk protein sequences from two monotreme species, platypus (*Ornithorhynchus anatinus*) and echidna (*Tachyglossus aculeatus*) and exhibit a full set of caseins and casein variants.

**Results:** The genomic organization of the platypus casein locus is compared to other mammalian genomes, including the marsupial opossum and several eutherians. Physical linkage of casein genes has been seen in the casein loci of all mammalian genomes examined and we confirm that this is also observed in platypus. However, we show that a recent duplication of beta-casein occurred in the monotreme lineage, as opposed to more ancient duplications of alpha-casein in the eutherian lineage, while marsupials possess only single copies of alpha and beta caseins. Despite this variability, the close proximity of the main alpha and beta casein genes in an inverted tail-tail orientation and the relative orientation of the more distant kappa casein genes are similar in all

mammalian genome sequences so far available.

**Conclusion:** Overall the conservation of the genomic organisation of the caseins indicates the early, pre-monotreme development of the fundamental role of caseins during lactation. In contrast, the lineage specific gene duplications that have occurred within the casein locus of monotremes and eutherians but not marsupials, which may have lost part of the ancestral casein locus, emphasises the independent selection on milk provision strategies to the young, most likely linked to different developmental strategies. The monotremes therefore provide insight into the ancestral drivers for lactation and how these have adapted in different lineages, including our own.

**Keywords:** Mammalian Lactation Milk, Evolution Transcriptomics

### **O-45: Quantification of Cell-Free-Fetal-DNA from Maternal Plasma for the First Time in Pakistan: Implications for Non-Invasive Prenatal Diagnosis of Genetic Disorders**

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**Background:** Current prenatal diagnosis requires invasive testing which carries a 1-4% procedure-related-risk of miscarriage; hence, non-invasive techniques are desired. The recent demonstration of cell-free-fetal-DNA enriched from maternal plasma has opened new possibilities for non-invasive-prenatal-diagnosis of not only genetic-disorders such as  $\beta$ -thalassaemia and haemophilia but also chromosomal-aneuploidy such as trisomy 21. Here, we report successful isolation of cell-free-fetal-DNA from maternal plasma as early as 12 gestational weeks for the first time in Pakistan.

**Materials and Methods:** Cell-free-fetal-DNA from 49 maternal plasma samples ranging from 12 to 41 weeks was analyzed for the SRY gene-sequence using quantitative-real-time-PCR. Male-genomic-DNA was used to prepare a four-point calibration-curve. Fetal gender confirmation was done at birth.

**Results:** From the 10 cases between 12-24 weeks, 2 male fetuses were observed with mean fetal DNA concentration of 15.86 (range: 11.96 – 19.76) GE/mL plasma; seven were determined to be female fetuses. From the 39 cases between 25-41 weeks, 10 male fetuses were identified with mean fetal DNA concentration of 268.4 (range = 0.85 – 1790.83) GE/mL plasma; twenty eight were identified as female fetuses. There was one case from each group which was reported as female fetuses; however, male gender was determined at birth.

**Conclusion:** We have demonstrated (1) 96% accuracy in fetal gender determination using maternal plasma as early as 12 weeks gestation; (2) that fetal DNA concen-

tration increased as pregnancy progressed. The lowest fetal DNA concentration quantified from maternal plasma was 0.85 GE/mL. Isolation of cell-free-fetal-DNA from maternal plasma provides an alternate access to fetal genetic material without invasive procedures. Potential genetic analysis in future includes assessment of sex-linked-disorders such as haemophilia, fetal RhD status, single-gene-disorders such as  $\beta$ -thalassaemia and chromosomal-aneuploidy such as trisomy 21.

**Keywords:** Cell-Free-Fetal DNA, Non-Invasive Prenatal Diagnosis, Maternal Plasma

### **O-46: The Expression of Leptin and Its Receptor during Different Physiological Stages in the Bovine Ovary**

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**Background:** Leptin, the hormonal product of the obese (ob) gene, circulates in the blood at levels paralleling those of fat reserves and regulates satiety. In cattle, leptin has also been implicated in the control of ovarian function, but its local production in the ovary and role in the control of ovarian function in autocrine/paracrine manner is unknown. The aims of this study were to document the expression of leptin and its receptor (Ob-R) in detail in bovine corpus luteum (CL) obtained from different stages of the oestrous cycle and during pregnancy and to determine if the leptin/Ob-R system is expressed clearly in bovine follicles during final growth to preovulatory follicles.

**Materials and Methods:** Experiment 1: corpora lutea (CL) were assigned to the following stages: days 1-2, 3-4, 5-7, 8-12, 13-16, and >18 of oestrous cycle and of gravidity (month <3, 3-5, 6-7 and >8). Experiment 2: Follicles during maturation were divided into granulosa cells (GC) and theca interna (TI) and were examined separately. Classification of follicles occurred by follicle size and oestradiol-17 $\beta$  (E2) concentration in the follicular fluid (FF) (<0.5 ng/ml, 0.5-5 ng/ml; 5-40 ng/ml; 40-180 ng/ml; >180 ng/ml). Real-time RT-PCR (qPCR) and ELISA were applied to investigate mRNA expression of examined factors and leptin protein respectively.

**Results:** In general, we demonstrated leptin and its receptor transcripts and leptin protein are consistent with *in vivo* luteinisation of bovine CL and decline coincidental with luteal regression. The highest co-expression of leptin/Ob-R system was observed in TI and GC of the smallest follicles with E2 concentration <0.5 ng/ml followed by significant down regulation in growing follicles with the increase of follicular size and E2 content in the follicular fluid. Furthermore, expression of the leptin/Ob-R system does not show any significant variation in

the CL throughout pregnancy.

**Conclusion:** Our results are the first to demonstrate the possible involvement of locally produced leptin/Ob-R system in the bovine ovary, suggesting roles in the function and/or development of the CL and growth of small follicles in an autocrine/ paracrine fashion.

**Keywords:** Leptin, Ob-R, Bovine Ovary, Follicle, Corpus Luteum

#### **O-47: Molecular Study of Internal Apoptotic Pathway BAX and BCL2 Genes and Mitochondrial Genome in Idiopathic Repeated Pregnancy Loss**

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**Background:** Pregnancy is the process from the fertilized ovum to the fetus with capability of extra uterine survival. Pregnancy loss is the most common complication of pregnancies. Advances in the detection of early pregnancy revealed that about 70% of human conceptions fail to achieve viability but clinically recognized pregnancies terminate as a miscarriage in about 15% of cases. About 1 in 300 couples and 0.5-2% of women are involved in repeated pregnancy loss (RPL). Various etiological factors involve in RPL and the main part of them remains unknown. Among them the genetic factors are important. The apoptotic changes and the aberrant expression of many genes including apoptotic related genes were seen in RPL.

**Materials and Methods:** Familial pedigrees of 335 consecutive couples suffering from RPL were initially evaluated at a primary stage. Among them, 96 women were screened as idiopathic at reproductive age. Molecular genetic variations in internal apoptotic related genes BAX, BCL2 and mitochondrial genome were investigated in comparison to control group. The methods were PCR-SSCP, PCR-Digestion-SSCP, Multiplex PCR and PCR-Direct sequencing.

**Results:** The evaluation of familial pedigree of 335 RPL couples showed 120 cases of RPL in female relatives and 76 cases in male relatives. Other families with RPL were seen in two or three consecutive generations in 15.6% of female relatives. At least two cases of RPL in other consanguineous marriages were observed in 4.2%. There were familial marriages in 51.6% of RPL women and 21.8% of control group ( $p=0.0003$ ). A statistically significant association was observed between the study and the control groups with regard to the frequency of alleles A to G (97.76% in RPL and 90.71% in control group) at nucleotide -179 in Bax promoter region ( $p=0.013$ ). G90C and G95A transitions were found in the coding region of exon 1

that change amino acid Glutamine (Q) to Histidine (H) and Arginine (R) to lysine (K) respectively. A statistically significant association was observed between H allele ( $p=0.0001$ ) and K allele ( $p<0.0001$ ) and the occurrence of RPL. Two nucleotide variations were seen in molecular analysis of Bcl2 gene. The G66C alteration in all RPL and normal women and A735G in 36.46% of RPL cases and 43.75% of control group ( $p=0.1449$ ). No deletions but a high frequency of point mutations were found in RPL females; some 129 variations were observed in RPL. The mean of the D-loop mutations was 8.79 and 4.90 in RPL and control group respectively (ANOVA=0.0001). Among them, 22 mutations were significant in RPL group and the insertion of C in nucleotide 114 was novel. D310 point mutations were seen in 51.04% of RPL women and 61.46% of control women. There were 4 variations in tRNA thr consisting of G15930A novel mutation. Also, a novel T15972C mutation was seen in tRNA pro.

**Conclusion:** The high frequency of RPL in maternal pedigree implicates the maternal background of the disorder. On the other hand, there were higher rate of familial repeats of abortion, RPL repeats and consanguineous marriage in RPL pedigrees. Such evidences show the genetic background. However, pedigree analysis has critical role in the approach of RPL women. Our result indicates a supportive role of RPL for A-179G mutation in Bax gene, but two polymorphisms, G90C and G95A found in exon 1, provide a susceptible background for promoting miscarriages. We believe that Bax gene has an important role in pregnancy loss. But, the Bcl2 alterations don't reflect any association in RPL. The RPL women did not have any deletions in mitochondrial genome. Deletions can lead to the early apoptosis, elimination of the cells and fetal loss. Although, we did not find any deletions in RPL women, investigating this issue in the aborted fetus as well could provide further information. High rates of mutations in D-loop of mtDNA were observed in maternal blood, a fact that may have a direct or indirect role in inducing RPL. There were 7 significant point mutations consisting of T16126C, T16189C, C16223T, C16294T, T16311C, T16362C, and T16519C more frequent in RPL females compared to the controls. Among 89 point mutations that were only detected in RPL group, C114 insertion was novel. Also, 15 variations consisting of T146C, C150T, C151T, T152C, T195C, T199C, C285T, C295T, C462T, T489C, C16069T, T16093C, C16148T, A16183C, and C16261T were significant in this group. These variations can have important roles in RPL, independently or as a part of haplogroups. The difference of D310 mutation between two studied groups was not significant. Disease-related point mutations could potentially influence mitochondrial tRNA and affect their primary, secondary, and tertiary structure. It leads to protein synthesis defects and, in turn, mitochondrial dysfunction. Ultimately, these disturbances result in cellular dysfunction which is more important in cell

proliferation and development. It seems more studies on these significant mutations can lead to the presentation of a diagnostic panel for RPL patients. Furthermore, preclinical abortions which are usually reported as infertile couples, and also failure of *in vitro* fertilization are our next targets for expanding this research to infertility.

**Keywords:** Repeated Pregnancy Loss, Apoptosis, Mitochondria, BAX, Bcl2

### **O-48: Heterogenous Spectrum of CFTR Gene Mutations in Indian Patients with Congenital Absence of Vas Deferens**

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**Background:** Mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene can cause congenital bilateral absence of vas deferens. Yet, the spectrum and frequency of CFTR mutations in Indian males with congenital absence of vas deferens (CAVD) is unknown.

**Materials and Methods:** We investigated 50 Indian males, diagnosed with unilateral or bilateral absence of vas deferens at the PGIMER, Chandigarh, for the presence of the most common CFTR gene mutations as well as unknown mutations by single-strand conformation polymorphism followed by sequence analysis.

**Results:** This study led to the identification of 12 CFTR gene mutations on 48% of 100 Indian CAVD chromosomes. CFTR mutations were identified on both alleles in 11 patients (22%) and on one allele in 26 patients (52%). Novel CFTR mutations identified were L69H, F87I, G126S, F157C, E543A, Y852F and D1270E. The T5 allele (25%) and F508del (11%) were the most common mutations identified. The most common intragenic marker haplotype for F508del was 2111 (GATT, TUB9, M470V and T854T). No mutations could be detected in 13 CAVD patients (26%), including 4 with renal malformations.

**Conclusion:** This study confirms the molecular heterogeneity of CFTR mutations in CAVD. Although the mutation detection rate is indeed lower in Indian CAVD patients, 74% of the patients tested had at least one CFTR mutation. CAVD alleles with no mutations suggest that other changes may be located at the non-screened sites that require extensive search by direct sequencing. Furthermore, the novel CFTR mutations identified require functional studies in a cell-based system.

**Keywords:** Congenital Absence of Vas Deferens, Cystic Fibrosis, CFTR, F508del, Single-Strand Conformational Polymorphism

## Poster Presentations

### Andrology

#### P-1: Leptin mRNA Is Present in Bovine Epididymal Spermatozoa

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**Background:** The present study aimed to investigate the presence of leptin mRNA transcript in epididymal spermatozoa of Holstein cattle by RT-PCR analysis.

**Materials and Methods:** To collect sperm from epididymis, the whole testes of three adult Holstein bulls were quickly removed after slaughtering. Samples were transferred to the laboratory on ice and subsequently the epididymal contents were collected and transferred to a plate containing BO medium. Then, normal and motile spermatozoa were isolated by swim-up method. Total RNA was extracted by TRIzol procedure, and was used to construct cDNA. The PCR with epididymal spermatozoa cDNA and outer leptin primer pairs resulted in amplification of the expected product. To confirm the first results, RT-PCR products were amplified with nested PCR using inner leptin primer pairs.

**Results:** Both outer and inner primer pairs, which were located in exon 2 and exon 3 be RNA specific, gave the expected PCR amplified products, 441 bp and 384 bp, respectively.

**Conclusion:** The presence of leptin mRNA in bovine epididymal spermatozoa suggest the ability of bovine sperm in leptin secretion, as established in human. Moreover, our results evoke the existence of a paracrine/autocrine mechanism for leptin in bovine sperm similar to human sperm and probable actions of leptin on bovine spermatozoa such as metabolism, capacitation and survival should be investigated.

**Keywords:** Bovine, Epididymal Spermatozoa, Leptin mRNA, RT-PCR

#### P-2: Impact of Sesame Oil Supplementation on Diabetic Rat Epididymal Sperm

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**Background:** Formation of hydrogen peroxide deriva-

tives due to metabolic disturbances in the male diabetics can affect their fertility. The antioxidant property of sesame oil has been documented, previously. The primary aim of the present study was to find if sesame oil supplementation can reduce the impact of diabetes on sperm parameters in rat or not.

**Materials and Methods:** Eighteen numbers of male Wistar rats assigned to four experimental groups: Control (n=3); intact animals received no treatment, Diabetic rats (n=5); diabetic rats treated with 5 (n=5) and 10% (n=5) of sesame oil within their diet throughout 60 days of experiment. Diabetes induced by IV injection of 45 mg/kg streptozotocin dissolved in citrate buffer (PH= 4.5). After 60 days of experiment, the animals were euthanized and their testes-epididymis dissected and weighted by digital balance. Left cauda epididymis were separated and total recovered sperms during 4 hrs of incubation in the normal saline (35- 37°C) were counted and divided by the weight of left testis and left epididymis to find total sperm/g testis and epididymis, respectively. Contra-lateral cauda epididymis dissected from testis and a sample of sperm isolated in normal saline (35-37°C). Sperm progressive motility (SPM) of recovered sperms was analyzed subjectively with light microscopy and analyzing four different fields of slides. Sperm viability was analyzed with conventional stain Eosin B/nigrosin stain. Data analyzed by ANOVA and expressed as Lsmeans ± ESM.

**Results:** Diabetes (228 ± 19.04 g) severely (p<0.05) reduced the animal weight compare to control (296 ± 19.04). Sesame extracts apparently (5 (242.2 ± 19.04) and 10 % (262.2 ± 19.04 g)) improved the impact of diabetes on animal weight (p>0.05). There was no significant difference between groups according to testes and epididymis weights (p>0.05). Surprisingly, total sperm/g testis and epididymis were not affected by diabetes (28.5 × 10<sup>6</sup>) (p>0.05) compare to control (28.7 × 10<sup>6</sup>) while they were severely influenced by sesame oil supplementation, either 5 (19.3×10<sup>6</sup>) or 10 (14.1×10<sup>6</sup>) %. Diabetes (19.4 ± 2.9) and 10 % supplementation of sesame oil (21 ± 2.9) severely affected (p<0.05) SPM compare to control (64 ± 2.9). Although, 5 % of sesame oil supplementation (37 ± 2.9) improved the SPM, it could not recover the SPM same as control (p<0.05). The percentage of viable sperms was not affected by different groups (p>0.05).

**Conclusion:** There are reports of beneficial effects of sesame oil on human health, mostly because of its biochemical components like sesaminol, which acts as a strong antioxidant. The results of the present study have shown the impact of sesame oil on sperm recovery from rat epididymis. The facts that sesame oil supplementation has adverse effects on the epididymal sperm in diabetic rats is related to metabolic disturbances of these kinds of rats or they have same effects on the intact animals is under investigation.

**Keywords:** Diabetes, Male Infertility, Sperm, Sesame Oil

### **P-3: Changes in Biochemical Parameters of Reproductive Function in Male Rats Infected with Toxoplasma Gondii**

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**Background:** Toxoplasma gondii is an intracellular protozoan with worldwide distribution capable of infecting all endothermic vertebrates. Latent toxoplasmosis in man and animals is considered as clinically asymptomatic. The aim of this Study is evaluation of the effects of Toxoplasma gondii on Biochemical Parameters of Reproductive Function in Male Rats.

**Materials and Methods:** A total of 56 male rats divided into infected group (IG) and control group (CG). The RH strain of T. gondii tachyzoites were injected interaperitoneally in an infected group of 35 rats, while 21 rats were used as controls. On days 10, 20, 30, 40, 50, 60 and 70 post-injection (PI), 5 rats from infected group and 3 rats from control group were anesthetized. The level of serum testosterone (ST), intratesticular testosterone (ITT), serum lactate dehydrogenase (S-LDH), intratesticular lactate dehydrogenase (IT-LDH) and fructose in seminal vesicles and coagulating glands (SVCG) was investigated.

**Results:** ST and ITT was significantly decreased on days 10 PI ( $p < 0.05$ ), S-LDH was significantly increased on days 10 PI ( $p < 0.05$ ), IT-LDH was significantly decreased on days 30 PI ( $p < 0.05$ ), and concentration of fructose in SVCG was significantly decreased on days 10, 20, 30, 40 and 50 PI ( $p < 0.05$ ).

**Conclusion:** According to the results, toxoplasmosis can cause changes in biochemical parameters of reproductive function in male rats.

**Keywords:** Biochemical Parameters, Reproductive Function, Male Rats, Toxoplasma Gondii, Toxoplasmosis

### **P-4: Adverse Effects Associated with Persistent Stimulation of Leydig Cells with hCG *In vitro***

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**Background:** The detrimental effects of persistent stimulation with hCG were investigated in rat Leydig cells *in vitro*.

**Materials and Methods:** Oxidative stress parameters such as lipid peroxidation, Reactive oxygen species

(ROS), Total antioxidant capacity (TAC) and activities of antioxidant enzymes were assessed in the treated cells. Apoptosis amongst the treated cells were detected by *in situ* end labeling (ISEL) assay. The increase in cell apoptosis was corroborated with the data on apoptotic markers such as caspase 3 activity, PARP cleavage, Fas, FasL, caspase 8, Bax, Bcl-2 and caspase 9.

**Results:** Significant rise in lipid peroxidation and ROS with concomitant attenuation in the activities of antioxidant enzymes; superoxide dismutase, catalase and glutathione-s-transferase was observed. Transcripts for catalase and superoxide dismutase were also found depleted. Subsequent to each hCG challenge, the total antioxidant capacity in the target cells also declined significantly ( $p < 0.05$ ). There was an increase in cell apoptosis (23%) which was associated with a rise in caspase-3 activity, PARP cleavage and Fas, FasL, caspase-8 expression. While Bax, Caspase-9 expression remained unchanged, Bcl-2 demonstrated a marked decline.

**Conclusion:** Taken together, the above data indicate that persistent hCG stimulation of Leydig cells induced adverse effects leading to oxidative stress and apoptosis which was channelled primarily through extrinsic pathway.

**Keywords:** Leydig Cells, Oxidative Stress, Apoptosis, Extrinsic Pathway

### **P-5: Chronic Mustard Toxicity on the Testis: A Historical Cohort Study Two Decades after Exposure**

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**Background:** We aimed to assess the long-term toxic effects of sulphur mustard (SM) on the testis and male fertility two decades after exposure.

**Materials and Methods:** A historical cohort study was conducted in 2005. Sixty-four SM-exposed and 64 matched SM-unexposed casualties of the Iraq-Iran conflict were enrolled. Fecundity status, semen indices, hormonal assay results and testis histopathology were evaluated.

**Results:** Male factor infertility was diagnosed in 23 and 5% of married exposed and unexposed casualties, respectively ( $p < 0.01$ ). All semen indices declined over the 15 years since 1990 among the exposed group. Furthermore, all indices with the exception of sperm motility were significantly lower in the exposed than in unexposed men. The follicle-stimulating hormone level was higher in the infertile than in fertile exposed men

( $p < 0.001$ ). Testis histopathology of the azoospermic men showed complete absence of spermatogenesis with only Sertoli cells in the seminiferous tubules.

**Conclusion:** SM can be gonadotoxic and its chronic toxicity may be permanent. Germ cells are probably the most susceptible gonadal cells to SM.

**Keywords:** Fecundity, Gonadotoxicity, Male Factor Infertility, Mustard, Semen, Testis

### **P-6: Beneficial Effects of $\alpha$ -Tocopherol Against Intracellular Calcium Overload in Normozoosperm**

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**Background:** Spermatozoa produce a variety of reactive oxygen species (ROS), including the superoxide anion and hydrogen peroxide. They are generated at very low concentrations in spermatozoa and association with signaling pathway and regulate physiological sperm function, for instance capacitation, acrosomal reaction, hyperactivation and sperm-oocyte fusion, the principal processes for fertilizing ability. In the physiologic condition there exists a subtle balance between ROS generating and scavenging systems for acquire of fertilizing ability. The capacity of antioxidant production is low in spermatozoa. An increasing in intracellular calcium induces acrosomal reaction (AR) and at the same time increases reactive oxygen species (ROS) production, which, in turn, enhances cell damage and cell death. This study was designed to determine the effect of an antioxidant ( $\alpha$ -tocopherol) on sperm motility and viability in control condition and in conditions which AR was induced by A23187 (a calcium ionophore).

**Materials and Methods:** Normozoospermic semen samples were obtained from 15 volunteers 20-30 years old after 3-5 days of sexual abstinence. Samples were washed, centrifuged and incubated in 37°C and 5% CO<sub>2</sub> until sperms swam up. Sperms were counted in the supernatant and divided into five groups, each contained 2×10<sup>6</sup> sperm/ml. Groups 1 to 5 were incubated for one hour with Ham's solution, 10 $\mu$ M A23187, 40 $\mu$ M  $\alpha$ -tocopherol, 10 $\mu$ M A23187 + 40 $\mu$ M  $\alpha$ -tocopherol, and ethanol, respectively in 37°C and 5% CO<sub>2</sub>. Sperm motility assessed by grading system ("a" to "d"), which is recommended by WHO.

**Results:** Our results indicated that,  $\alpha$ -tocopherol has ability to prevent sperm mortality and save sperm rapid motility after 1 hour incubation. In the same time, A23187 reduced significantly percent of rapid sperm motility and

increased sperm mortality. Results of sperms incubation in the medium contain a combination of A23187 and  $\alpha$ -tocopherol showed that  $\alpha$ -tocopherol can reduce many of deleterious effects of A23187.

**Conclusion:** Addition of  $\alpha$ -tocopherol to sperm media prevents the decrement of rapid sperm motility as well as mortality. It seems that the harmful effects of A23187 are due to excessive ROS production and  $\alpha$ -tocopherol neutralizes these effects.

**Keywords:** Sperm Motility, Vitality,  $\alpha$ -Tocopherol, A23187, Reactive Oxygen Species

### **P-7: Association of Gonad Failure with Serum Insulin Level**

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**Background:** Serum insulin variations are occasionally observed in patients suffering infertility or gonad failure. The main purpose of the present study was to clarify the effects of gonadectomy and sex steroid hormone replacement on serum insulin level in rats.

**Materials and Methods:** Male rats were divided into control, orchidectomized, and testosterone receiving orchidectomized animals. Female rats were also divided into control, ovariectomized, progesterone or estradiol receiving ovariectomized animals. Testosterone (50mg/kg/day) or progesterone (20 mg/kg/day) and estradiol (200 $\mu$ g/kg/day) were injected intraperitoneally or subcutaneously, respectively. The period of 4 weeks was considered for each experiment. After 4 weeks, serum insulin was measured and compared statistically between the groups (ANOVA).

**Results:** In male rats, orchidectomy caused to decreasing ( $p < 0.001$ ) but testosterone replacement resulted in increasing of serum insulin level ( $p < 0.01$ ). In female rats, ovariectomy caused to decreasing of serum insulin ( $p < 0.001$ ) and estradiol replacement prohibited but progesterone replacement could not prohibit the decreasing of insulin secretion after ovariectomy ( $p < 0.001$ ).

**Conclusion:** our findings indicated that sex steroid hormones play a pivotal role in insulin secretion. Therefore, in patients suffering infertility or gonad failure caused by gonadal hormones disorders, insulin pathophysiology should be considered clinically.

**Keywords:** Insulin, Orchidectomy, Ovariectomy

### **P-8: Males' Infertility Assessment Using Semen Analysis**

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**Background:** Sperm analysis is the first step to evaluate and to diagnose males infertility. Although, it is less complicated than women's infertility, however, it can account up to 40% of infertility rate in the community. Total analysis of density, vitality and motility percentiles are the important ways evaluating men's' infertility.

**Materials and Methods:** This was a case-control study of 96 men attended to the infertility clinics in Ilam province from May 2008 to May 2009. Demographic characteristics were collected using a validated questionnaire. Urological examinations were undertaken for all enrolled samples. Following sperm collecting, semen analysis was done using the Weili Dynamic Sperm Analysis software adapted to WHO classification. Based on movement and speed characters, sperms are classified to either A, B, C or D classes and subjects were stratified into two groups of "cases" called "Oligospermia" with sperm counts of less than 20 million in mL (n=48) and "controls" called "Non-oligospermia" with values more than determined cutoff point (n=48). Both groups were matched according to occupation, age, smoking and exposure to radiation.

**Results:** The Mean age $\pm$  standard deviation for cases and controls were 29.9  $\pm$  5.1 years and 31.17  $\pm$  5.24 years respectively. The Mean BMI was 25.3 and 25.1 kg/m<sup>2</sup> in cases and controls respectively with no significant relationship. Overall, 62.5% of cases and 31.2% of controls were clinically infertile (OR=3.6, CI, 1.5-8.5, p=0.01). There was a significant association between job categories (militaries and workers) and male's infertility in both groups (p<0.002). There was an inverse correlation between mean age and Live Ratio (Class A+B+C), (r=-0.417, p<0.002).

**Conclusion:** Getting old was associated with infertility amongst certain employees in particular. Surprisingly, no significant relationship was observed between BMI and infertility. Further case-control studies and clinical trials are recommended to recognize infertility causes in men definitively.

**Keywords:** Infertility, Weili Dynamic, Sperm Analysis, Men

### P-9: Correlations between Age, Charlson Score and Outcome in Clinical Unilateral T3a Prostate Cancer

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**Background:** According to the EAU guidelines, a life expectancy of more than 10 years is considered an important factor in the treatment of prostate cancer. The Charlson score is used to predict mortality based on co-

morbidity. The purpose of this study was to investigate the relationship between age, Charlson score and outcome in patients with cT3a prostate cancer.

**Materials and Methods:** Between 1987 and 2004, 200 patients with clinical unilateral T3a prostate cancer, detected by digital rectal examination, underwent radical prostatectomy (RP). Patients were categorized into two age groups (<65 and  $\geq$ 65 years old). Patients were also divided into two groups according to Charlson score (score = 0 and  $\geq$ 1). Both age and Charlson score were analyzed regarding their predictive power in patient outcomes.

**Results:** The mean follow-up was 70.6 months, and the mean age was 63.3 years. One hundred and six patients were younger than 65, and 94 patients were  $\geq$ 65 years old. Age was a significant predictor in overall survival (OS). A Charlson score of 0 was found in 110 patients, and a Charlson score  $\geq$ 1 in 90 patients. Charlson score was not a significant predictor of biochemical progression free survival (BPFS), clinical progression free survival (CPFS) or OS. Cox multivariate analysis revealed that margin status was a significant independent factor in BPFS, and cancer volume was a significant independent factor in CPFS.

**Conclusion:** Charlson score does not influence outcome in patients with clinical locally advanced prostate cancer. Age may influence OS. RP can be performed in motivated healthy older patients. However, the patients need to be counseled regarding possible surgery-related side effects, such as urinary incontinence and erectile dysfunction, which are age- and comorbidity-dependent.

**Keywords:** Age, Charlson Score, cT3a Prostate Cancer

### P-10: An Experimental Design in Rats-Anti Inflammatory Drug and Male Infertility

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**Background:** In some patients that used celecoxib for a long time, unusual effect of this drug may be seen. Celecoxib is a nonsteroidal anti-inflammatory drug. This is a selective Cox-2 inhibitor. Nowadays this drug uses as an analgesic, antipyretic and anti-inflammatory agent frequently. The goal of this survey is assess the effect of this drug on male-reproductive system functions

**Materials and Methods:** This survey done for study of effect of celecoxib on rat reproductive system, specially on spermatogenesis and the level of blood testosterone hormone.

In this manner histologic studies and measuring of weight (testis, prostate, seminal vesicle and epididymis) and the level of blood testosterone are done.

50 rat with 200-230 gr. 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 40mg/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 no significant differences in mean weight of body testis, epididymis and seminal vesicle in control and case groups. But significant differences are seen in the mean weight of prostate per body weight in case group (40 mg/kg) in compared with control group. That is due to ant malignant effect of celecoxib on prostate.

No differences seem between control and case groups in arrangement mode, nuclei shape and cytoplasm in histological examination in spermatogonia and primary spermatocytes in transverse section of seminiferous tubules celecoxib in case group (40 mg/kg) can decrease lydig cells number, that is due to inhibited prostaglandin synthesis, for the result of cox-2 inhibitor and decreased level of testosterone hormone.

**Conclusion:** It looks that number of 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 testosterone level. This can cause production of abnormal sperms.

In the survey can conclude that use of high doses of celecoxib can decreased size and number of lydig cells and this is cause of decreased testosterone hormone.

**Keywords:** Celecoxib, Male, Infertility, Testosterone Hormone

### **P-11: Exogenous Melatonin Protects Folliculogenesis and Endometrium from Apoptosis in Female Mouse Treated with Nicotine**

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**Background:** Nicotine exposure causes impaired fertility, ovarian and uterus dysfunction. The pineal hormone melatonin is known as an antioxidant agent. The aim of this study was to investigate the possible protective role of exogen melatonin on altered ovary and endometrium with nicotine.

**Materials and Methods:** Female adult NMRI mice were divided into four groups. The control group received vehicle, group 2 received nicotine (0.4 mg/100g body weight) for 15 days, group 3 was administered melatonin (10 mg/kg) for 5 days. Group 4 received both nicotine (0.4 mg/100g body weight) and melatonin (10 mg/kg). All animals were treated intraperitoneally. After autopsy on 16th day, evaluations were made by histopathology and in situ TUNEL assay. Statistical analysis were performed using ANOVA test.

**Results:** Nicotine significantly reduced the number and size of pre-antral and antral follicles compared to the control. ( $p < 0.01$ ). However the numbers of primordial follicles in nicotine- treated group were reduced but it was not significant. Both ovaries ( $11.8 \pm 0.03$  Vs  $3.6 \pm 0.01$ ) and endometrium ( $6.8 \pm 0.04$  vs  $1.4 \pm 0.02$ ) of mice exposed to nicotine had a significant increase in the percentage of apoptotic cells compared to controls, while melatonin in group 4 caused a marked normalization in number and size of ovarian follicles compared to group 2 ( $p < 0.01$ ). The percentage of apoptotic cells in ovary significantly reduced in group 4 compared to the group 2 ( $6.2 \pm 0.01$  vs  $11.8 \pm 0.03$ ). Melatonin also reduced apoptotic cells in endometrium compared to group 2 ( $2.3 \pm 0.06$  vs  $6.8 \pm 0.04$ ).

**Conclusion:** The results from this study suggest that melatonin may have a protective effect against nicotine-induced ovarian and endometrium damage through reduction of apoptosis and probably by decreasing oxidative stresses.

**Keywords:** Apoptosis, Endometrium, Melatonin, Nicotine, Ovary

### **P-12: Effects of Herbal Drug NOFODA on Sperm Parameters of Infertile Men**

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**Background:** Treating infertility doesn't merely deal with advanced medical procedures. The effects of most of herbal drugs is not clearly established on fertility potential. One of this herbal formula is NOFODA comprising of Orchis mascula, Tribulus terrestris, Amygdalus communis, Ficus carica, Allium ampeloprasum, Nasturtium officinale and pollen of Phoenix dactylifera. In this study, the effects of NOFODA on count, morphology and motility of sperms was investigated in subjects with male infertility.

**Materials and Methods:** Trial was performed with 60 infertile men (age  $30.40 \pm 5.21$  years). Half of the patients received 500 gr oral NOFODA, 3 times a week for 3 months. The sperm parameters were evaluated according to WHO criteria before and after treatment, then compared with control. The NOFODA administration data were analyzed using t test by SPSS 16.0.

**Results:** The results revealed that progressive motility (a+b class) and sperm count improved from  $37.60 \pm 16.13$

and  $59.93 \pm 42.91$  to  $48.06 \pm 19.63$  ( $p < 0.001$ ) and  $79.86 \pm 62.05$  ( $p < 0.05$ ), respectively. However, no significant increase was detected in sperm morphology (from  $18.83 \pm 10.59$  to  $20.00 \pm 12.94$ ). The sperm parameters of count and progressive motility were increased significantly after 3 months of treatment in case group when compared with control.

**Conclusion:** It seems that NOFODA is positively effective on sperm parameters of infertile men. Administration of this natural herbal drug is recommended in management of infertility.

**Keywords:** Sperm Parameters, Male Infertility, Herbal Formulation, WHO

### **P-13: Male Reproductive Organs Are at Risk from Environmental Hazards**

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**Background:** Male reproductive disorders that are of interest from an environmental point of view include sexual dysfunction, infertility, cryptorchidism, hypospadias and testicular cancer. Several reports suggest declining sperm counts and increase of these reproductive disorders in some areas during some time periods past 50 years.

**Materials and Methods:** Except for testicular cancer this evidence is circumstantial and needs cautious interpretation. However, the male germ line is one of the most sensitive tissues to the damaging effects of ionizing radiation, radiant heat and a number of known toxicants. So far occupational hazards are the best documented risk factors for impaired male reproductive function and include physical exposures (radiant heat, ionizing radiation, high frequency electromagnetic radiation), chemical exposures (some solvents as carbon disulfide and ethylene glycol ethers, some pesticides as dibromochloropropane, ethylenedibromide and DDT/DDE, some heavy metals as inorganic lead and mercury) and work processes such as metal welding.

**Results:** Improved working conditions in affluent countries have dramatically decreased known hazardous workplace exposures, but millions of workers in less affluent countries are at risk from reproductive toxicants. New data show that environmental low-level exposure to biopersistent pollutants in the diet may pose a risk to people in all parts of the world. For other toxicants the evidence is only suggestive and further evaluation is needed before conclusions can be drawn. Whether compounds as phthalates, bisphenol A and boron that are present in a large number of industrial and consumer products entails a risk remains to be established. The same applies to psychosocial stressors and use of mobile phones.

**Conclusion:** Finally, there are data indicating a particular vulnerability of the fetal testis to toxicants—for instance maternal tobacco smoking. Time has come where male

reproductive toxicity should be addressed from entirely new angles including exposures very early in life

**Keywords:** Environmental Hazards, Male Infertility

### **P-14: Effect of Oxcarbazepine on Male Reproductive Physiology**

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**Background:** Oxcarbazepine is a widely used novel antiepileptic drug. In vitro electrophysiology studies indicate that oxcarbazepine produced blocked of voltage-sensitive sodium channels, resulting in stabilization of hyper excited neural membranes, inhibition of propagation of synaptic impulses. In this research the effect of oxcarbazepine has been studied on the amount LH, FSH, testosterone Hormones and male Reproductive physiology.

**Materials and Methods:** In this research 40 adult male was stricken to epilepsy used accidentally between the conferrings to neurological clinics of shiraz in four groups which each of them was consist of 10 members, was as follow: Control group not received each material and experimental groups was received 150,300,600 mg/day amount of oxcarbazepine as oral (tablet) for 30 days and after the termination of this period for measuring the amount LH, FSH, testosterone Hormones of blood shooting from cephalic vessels of hand. And obtaining result was analyzed, by using ANOVA, Duncan and Tukey Test.

**Results:** According to the obtained result, the in plasma concentration of LH presented a significant increased at  $p < 0.05$ , whereas in plasma concentration of FSH weren't seen any significant change. However, in plasma concentration of testosterone presented a significant decrease at  $p < /05$  in using of oxcarbazepine on three experimental groups of min (150 mg/day), med (300 mg/day) and max (600 mg/day) observed that the amount of male reproductive potential decrease with the increasing of drug dose.

**Conclusion:** Obtaining results demonstrated that use up of oxcarbazepine can have negative effect on leydig cells in testis and the production of testosterone was decrease and for this reason the Hypothesis axis is decided to compensate this reduction with more decrease of LH.

**Keywords:** Oxcarbazepine, Reproductive Potential, Sexual Hormones

### **P-15: Study Of the Effect of Nicotine - Melatonin on Sperm Parameters in Adult Mouse**

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**Background:** Cigarette smoking is associated with impairment of testicular function. Nicotine is a major component of cigarette smoke. In the present study we aimed to study the effect of administration of nicotine-melatonin on sperm parameters in adult mouse.

**Materials and Methods:** Male NMRI mice were divided into 4 groups: 1) control (normal saline) group 2) nicotine treated group 3) melatonin treated group 4) Nicotine-melatonin treated group. Nicotine was administered in dose of 0.04mg/100g.bw (equivalent to heavy smokers) intraperitoneally for 14 days. Melatonin was administered in dose of 10 mg/kg for 5 days intraperitoneally. On 15 days after the treatment sperm samples were obtained from cauda epididymis. Parameters measured were sperm count, abnormal head and tail morphology and sperm motility. Motility was scored as fast progressive, slow progressive, shaking and immotile sperms. Statistical analysis was performed by ANOVA test

**Results:** Nicotine caused a decrease in sperm count ( $p < 0.001$ ) and percent of fast progressive motile sperms ( $p < 0.03$ ) while increasing slow progressive motile sperm and abnormal tail ( $p < 0.001$ ). However percent of abnormal head, shaking or immotile sperms were not affected. Melatonin alone, had no effect on sperm count and morphology however, significantly reduced motility in comparison with control ( $p < 0.05$ ). Administration of nicotine-melatonin increased sperm count and reduced abnormal sperms ( $p < 0.05$ ) but motility rate were not affected in comparison with nicotine treated group.

**Conclusion:** These findings suggest that administration of 0.04mg/100g.bw nicotine for 14 days leads to a deficiency in sperm parameters. Melatonin may have a protective effect on sperm count and morphology against nicotine without effect on motility, probably by reducing the oxidative stresses.

**Keywords:** Melatonin, Nicotine, Sperm Parameters

**P-16: Lifestyle Factors in Deteriorating Male Reproductive Health**

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**Background:** Many health problems are related to lifestyle and dietary factors. Increasing trend in reproductive disorders observed in recent years may be associated at least in part with these factors, which are compounded by some of the new emergent life styles.

**Materials and Methods:** The data available suggests that lifestyle factors such as obesity, tobacco smoking or chewing, alcohol and some of the illicit drugs like co-

caine, cannabis etc and exposure to extreme heat, have adverse effects on male reproduction.

**Results:** The data on other factors such as use of mobile phone and stress on reproductive health are inadequate and need detailed study.

**Conclusion:** Lifestyle related diseases could be lowered with modification in diet, living and working environment etc. Sub-fertile and/or normal subjects have some control over their reproductive function by adopting healthy lifestyles to avoid additional complications

**Keywords:** Lifestyle Factors, Male Reproductive Health

**P-17: Modulation of Wnt/ $\beta$ catenin and Akt Signaling Activities in Rat Granulosa Cells by Using Recombinant Secreted Frizzled Related Protein Type-4**

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**Background:** Secreted frizzled-related protein-4 (sFRP-4) belongs to a family of soluble proteins that have a Frizzled-like cysteine-rich domain and function as modulators of Wnt signaling and it also appears to antagonize a molecular pathway for cell survival. Of interest to our studies, sFRP-4 has been found to be expressed in the rat ovary but its role in Wnt/ $\beta$ -catenin modulation and AKT/PKB activity remains unknown. The aims of this study were two-fold. Firstly, we sought to determine in greater detail the activation of AKT/PKB as a cell survival marker. Secondly, the study investigated whether sFRP-4 may modulate Wnt/ $\beta$ -catenin by assessment of GSK3- $\beta$  activity and stabilized  $\beta$ -catenin as downstream proteins of Wnt signaling.

**Materials and Methods:** Follicular growth was hormonally induced in immature Wistar rats by using PMSG and their ovaries collected after 48 hours. Granulosa cells were harvested mechanically and cultured with testosterone (0.1nM) and recombinant human FSH (50 ng/mL) in the presence or absence of recombinant human sFRP-4 (rhsFRP-4) (0.5-50 ng/ml) for 48 hours. These cells were named as FSH-primed cells and were subsequently treated with ovine LH (500 ng/mL) or rhsFRP-4 alone or both in combination for another 48 hours. In addition few experiments were designed by using rhsFRP-4 three hours prior of LH treatment. After four days, granulosa cells were lysed for western blot analysis of stabilized  $\beta$ -catenin known as active  $\beta$ -catenin, AKT/PKB and GSK3 $\beta$  activities.  $\beta$ -actin assessment was used as an internal control and Results were quantified by scion software.

**Results:** FSH-primed cells treated with low dose of rhsFRP-4 showed increased GSK3 $\beta$  ( $1.6 \pm 0.17$  fold,  $p < 0.05$ ) activity as revealed by detection of GSK3 $\beta$

phosphorylated in serine 9 position which was further accompanied with increased levels of activated  $\beta$ -catenin ( $2.8 \pm 0.2$ ,  $p \leq 0.05$ ). In contrast, treatment of FSH-primed cells with high dose of rhsFRP-4 caused decreased levels of GSK3 $\beta$  and  $\beta$ -catenin activities. Interestingly, FSH-primed cells treated with LH showed significant increased levels of AKT/PKB activity as revealed by detection of AKT/PKB phosphorylated in serine 473 which may be abrogated by use of rhsFRP-4 prior to LH stimulation.

**Conclusion:** Our results could suggest that Wnt/ $\beta$ -catenin signaling may mediate the effect of FSH on granulosa cells which may be modulated by sFRP-4 in a dose dependent manner. Moreover, LH-induced Wnts in rodent ovary may be involved in granulosa cell survival which could be reduced in the presence of sFRP4. Thus, sFRP-4 may modulate both Wnt/ $\beta$ -catenin and AKT/PKB pathways in granulosa cells depending on the differentiated stage of these cells.

**Keywords:** Recombinant Human sFRP-4, GSK3- $\beta$ , Wnt/ $\beta$ -Catenin Signaling, Akt/PKB Pathway, Rat Ovarian Granulosa Cells

### **P-18: Effect of Forced Swimming Stress on Count, Motility and Fertilization Capacity of the Sperm in Adult Rats**

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**Background:** The purpose of this study was to determine whether 50 days of forced swimming stress applied to adult male rats affects count, motility and fertilization capacity of sperm

**Materials and Methods:** A total 30 adult male wistar rats were used in this study. All rats were divided into two equal groups ( $n = 15$ ): (1) control group and (2) experimental group. Animals of the experimental group were submitted to force swimming stress for 3 min in water at 32°C daily for 50 days. Then, all male rats were sacrificed, the right epididymides were removed and sperm concentration and motility were determined. The sperm suspension was added to the ova. Fertilization capacity was assessed by counting two-cell embryos 24-26 h after completion of fertilization in vitro. Statistical Analysis Used: Data are reported as mean  $\pm$  SD and percentage. The difference between the control and experimental groups was determined by the unpaired t-test

**Results:** The mean and standard deviation of sperm concentration in the control and experimental groups were  $60.8 \pm 9.3 \times 10^6$  /ml and  $20.4 \pm 5.3 \times 10^6$  /ml, respectively. There was a statistical difference of  $p < 0.05$  between the two groups in terms of sperm concentration. The percentage of motility in the experimental group was significantly different ( $p < 0.05$ ). The same results were obtained in case of fertility ( $p < 0.05$ ). Stress caused by forced swimming was observed by a significant increase

in the latency of the pain response in the hot-plate test ( $p < 0.05$ )

**Conclusion:** These results suggest that forced swimming stress in time course equal or more than spermatogenesis period, i.e. 48-50 days in the rat will be significantly effective to reduce the number and motility of sperms as well as the fertilization capacity.

**Keywords:** Stress, Fertilization, Sperm Motility

### **P-19: Effect of Hydro Alcoholic Leaf Extract of Myrtus Communis on Pituitary- Gonad Hormonal Axis in Adult Male Rat**

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**Background:** Myrtus communis has been identified as a holy plant for ages and a great variety of medicinal properties have been described for it in literature. Today, there is a number of Myrtus drugs in Iran market including: Myrtoplex cream, Myrtex Solution, Aftoplex, Dineh Inhaler Powder, Rectol cream and so on. These properties may due to the presence of different chemical compounds such as Tanin, Flavinoid, Saponin, Ascorbic acid, 1,8-Cineole, Myricetin and Delta-cadinene. Since Myrtus communis is used to treat sexual impotent in some areas, present study was performed to evaluate the effect of hydro alcoholic Myrtus communis leaf extract on pituitary – gonad axis in adult male rat.

**Materials and Methods:** Forty adult male rats wistar strain were selected and randomly divided into five groups; control ( $n=8$ ) which received no treatment, sham which received distilled water as a solvent ( $n=8$ ), and three experimental groups (1,2 and 3) ( $n=8$  for each of group) which received 0.75, 1.5 and 3 mg/kg Myrtus communis leaf extract respectively. The extract received by orally and the test period was 21 days. After the latest prescription of extract, the animal anaesthetize and give blood from their haert. Then considered the concentration of FSH, LH and testosterone by radio immunoassay (RIA) method. The obtained results analyzed based on SPSS, Excel, analysis one –way variance and post hoc statistical programs, and significant ( $p < 0.05$ ) take in consideration.

**Results:** The results of hormonal examination indicated the amount of 1.5 and 3 mg/kg body weights of myrtus communis leaf extract showed a significant increase in the level of testosterone ( $p < 0.05$ ) but concentration of LH, FSH hormones showed no significant difference.

**Conclusion:** The Myrtus communis leaf extract causes an increasing testosterone hormone, that probably related with the compounds as Flavinoid, Ascorbic acid and Myricetin (by inhibition of aromatase activity), Linoleic

acid, Oleic acid and Palmitic acid (by inhibition 5alpha reductase activity) and 1,8-Cineole, Delta-cadinene (the cytochrome-P450 Inducer).

**Keywords:** Myrtus Communis, Testosterone, Rat

## **P-20: The Relationship between Occupation and Semen Quality**

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**Background:** The main object 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 men who attended the Infertility Clinic of Royan Institute, located in Tehran, Iran. The study consisted of questionnaires completed by trained interviewers to provide information about demographics marital status, type and duration of infertility, occupational history including job title and task, and exposure to occupational physical hazards. Additionally, medical examinations by an urologist and semen analysis tests were performed for each participant. A total of 1164 patients were recruited by simple randomization from September 2009 to March 2010. Each subject signed an informed consent document after the goals of the study were fully explained. For statistical analysis SPSS 16 for Windows was used. Descriptive statistics were used to characterize the study population. The relationships between semen parameters, age and body mass index (BMI) were investigated using Spearman non-parametric correlation. Analysis of variance was used to compare semen parameters between occupational groups.

**Results:** The means and standard deviations of age, infertility duration and BMI of the participants were 33.83 ± 5.79 years, 6.23 ± 0.14 years and 26.1 ± 4.12, respectively. The frequencies of primary and secondary infertility were 89.7 and 10.3 percent, respectively. Sperm analysis test results revealed that the means and standard deviations of the sperm count, percentage of normal sperm morphology and percentages of sperm with class A and class B motilities were 44.89 ± 32.43 million/ml, 7.36 ± 5.3, 8.6 ± 7 and 23.13 ± 10.8, respectively. Statistical analysis revealed a significant negative correlation between age and the mean percentage of sperm with

Class B motility ( $r_s = -0.13$ ,  $p < 0.001$ ), but there were no statistically significant correlations between age and other sperm parameters, nor between BMI and sperm parameters. 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. Also, heavy physical exertion and prolonged sitting were reported in 43.7% (n=509) and 62.1% (n=723) of participants, respectively. According to participants' occupations and considering similar occupational exposures, twelve occupational categories were derived: Office work, Transportation, Construction, Sales, Services, Agriculture, Metal working, Plastic working, Painting, Mechanical trades, Armed forces and Electronics. The four most common occupational categories among participants were Office work, Sales, Transportation and Construction with frequencies of 30, 13.8, 10.1 and 10 percent, respectively and the least common was Plastic working with a frequency of 1.7 percent. There were no statistically significant differences in the mean sperm count nor the sperm morphology between occupational categories. Assessment of the differences in the frequency of the sperm motility classes between occupational categories revealed a significant difference only in the frequency of sperm with Class B motility ( $p = 0.03$ ). The highest and the lowest mean percentages of sperm with Class B motility were seen in the Electronics (mean ± SD: 25.94 ± 12.3) and the Transportation (mean ± SD: 20.26 ± 10.6) categories, respectively.

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

**Keywords:** Occupational Category, Risk Factors, Semen Quality

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## **Embryology**

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### **P-21: Sodium Selenite Improves the *In vitro* Follicular Development by Reducing the Reactive Oxygen Species Level and Increasing the Total Antioxidant Capacity and Glutathione Peroxide Activity**

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**Background:** The aim of this study was to investigate the compare of Conventional (CV) and Direct Cover (DCV) vitrification and effect of sodium selenite(SS) that supplemented with FBS and BSA on reactive oxygen species (ROS) production, total antioxidant capacity (TAC) and glutathione peroxidase (GPx) activity of cultured pre-antral follicles derived from vitrified and non-vitrified ovarian tissue.

**Materials and Methods:** Immature mouse ovaries were vitrified (conventional and direct cover methods), and mechanically isolated pre-antral follicles from vitrified and non-vitrified samples were cultured in TCM 199 medium with FBS and BSA and supplemented with different concentrations (0, 5 and 10 ng/ml) of SS. Follicular, oocyte and embryo development was assessed. In parallel, ROS, TAC and GPx levels were analyzed after 0, 12, 24, 48, 72 and 96 h of culture.

**Results:** The normality of primary and preantral follicles in CV groups were higher than those achieved by DCV groups ( $p < 0.001$ ). Development rates of follicles, oocytes and embryos were significantly higher in SS-supplemented groups ( $p < 0.005$ ). ROS production was increased, and TAC levels and GPx activities were decreased after 24 h of culture of pre-antral follicles in vitrified and non-vitrified groups, whereas in the presence of SS, ROS production was decreased and TAC levels and selenium-dependent GPx-specific activities were increased after 96 h of culture. Vitrified and non-vitrified samples responded in a similar manner.

**Conclusion:** DCV of mouse ovarian tissue using only EG has induced some alteration on the fine structure of follicles and the sodium selenite and FBS improve the in vitro growth and maturation of mouse preantral follicles. SS caused an increase in follicular TAC level and GPx activity and a decrease in ROS level, thus improving the in vitro development of follicles.

**Keywords:** Ovarian Tissue, Pre-Antral Follicles, Reactive Oxygen Species, Selenium, Vitrification

### **P-22: Effect of Bovine Cumulus Cell Co-Culture on Maturation of Buffalo (*Bubalus Bubalis*) Oocyte**

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**Background:** The buffalo (*Bubalus bubalis*) is the animal with special production characteristics in producing milk and meat has a critical role in rural industry in Iran. The most problem of this specie is its low reproductive output. The aim of the present study was to evaluate the effect of bovine cumulus co-culture on maturation rate of Buffalo oocyte.

**Materials and Methods:** Ovaries were collected from slaughtered buffaloes. The cumulus oocyte complexes (COC) were picked up from follicles (2-8 mm). The grade A and B COCs were randomly divided into 3 groups. Group 1; COCs were subjected to oocyte maturation medium containing TCM-199+ 5%FCS + 5%FF + 10µg/ml LH + 10 µg/ml FSH. Group 2; the COCs were cultured on a monolayer culture of bovine cumulus cells in a medium contained TCM199 with 5% FCS. Group 3; the denuded COCs were subjected on a monolayer culture of bovine cumulus cells in a medium similar to group 2. After 24 h of maturation in an atmosphere with 5 % CO<sub>2</sub> and 95% relative humidity, the nuclear maturation of the oocytes was evaluated using a conventional aceto-orcein stain.

**Results:** The maturation rate was significantly ( $p < 0.05$ ) improved after incubation of COCs in monolayer culture of bovine cumulus cells (81.3 %) compare to control and the denuded co-cultured buffalo oocytes (66.66 and 70 %, respectively;  $p > 0.05$ ).

**Conclusion:** The results of the present study indicate the high efficacy of bovine cumulus cell monolayer on maturation of buffalo oocytes.

**Keywords:** Cumulus Monolayer Co-Culture, Buffalo Oocyte, Maturation

### **P-23: Assessment of Growth Factors Effect on Post-Thaw Development of Mouse Two-Cell Embryo to Blastocyst Stage after Vitrification**

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**Background:** The aim of this study was to assess the influence of in-vitro culture system supplemented with specific growth factors on post-thaw development of mouse vitrified 2-cell embryos.

**Materials and Methods:** 6-8 weeks old female NMRI mice were superovulated with 5IU pregnant mare,s (PMSG,ip) and subsequent human chorionic gonadotrophin (hCG, ip).mated mice were killed vy cervical dislocation to collect 2-cell stage embryos from oviduct of pregnant 2 d mice. After vitrification of embryos by cryotop, thawed 2-cells embryos (n= 166)were randomly distributed among 3 groups: medium alone control (T6, I), T6 medium supplemented with 20 ng/ml of fibroblast growth factor (FGF, II) and 20 ng/ml of hepatocytes growth factor (HGF, II) and were cultured to blastocyst stage.

**Results:** Cell proliferation and blastocyst development rate were significantly stimulated by exposure to growth factors (I and II groups) in comparison with control

group ( $p < 0.05$ ). There was not significant difference in the survival rate of thawed embryos among the control and treated groups ( $p > 0.05$ ).

**Conclusion:** Culture of thawed 2-cell embryos in medium supplemented with FGF and HGF seems to be a useful way to eliminate the post-thaw deleterious affect of vitrification and also to obtain better-quality embryos appropriate for transfer.

**Keywords:** Vitrification, HGF, FGF, Embryos Development, Cryotop

### **P-24: Opioid and Progesterone Signaling Is Obligatory for Early Human Embryogenesis**

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**Background:** The growth factors that drive the division and differentiation of stem cells during early human embryogenesis are unknown. The secretion of endorphins, progesterone (P(4)), human chorionic gonadotropin, 17beta-estradiol, and gonadotropin-releasing hormone by trophoblasts that lie adjacent to the embryoblast in the blastocyst suggests that these pregnancy-associated factors may directly signal the growth and development of the embryoblast.

**Materials and Methods:** To test this hypothesis, we treated embryoblast-derived human embryonic stem cells (hESCs) with ICI 174,864, a delta-opioid receptor antagonist, and RU-486 (mifepristone), a P(4) receptor competitive antagonist.

**Results:** Both antagonists potently inhibited the differentiation of hESC into embryoid bodies, an in vitro structure akin to the blastocyst containing all three germ layers. Furthermore, these agents prevented the differentiation of hESC aggregates into columnar neuroectodermal cells and their organization into neural tube-like rosettes as determined morphologically. Immunoblot analyses confirmed the obligatory role of these hormones; both antagonists inhibited nestin expression, an early marker of neural precursor cells normally detected during rosette formation. Conversely, addition of P(4) to hESC aggregates induced nestin expression and the formation of neuroectodermal rosettes.

**Conclusion:** These results demonstrate that trophoblast-associated hormones induce blastulation and neurulation during early human embryogenesis.

**Keywords:** Progesterone, Opioid, Blastulation, Neurulation, Nestin, Embryogenesis

### **P-25: Role of Oocyte Morphology on ART Outcome**

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**Background:** The cause of fertilization failure in IVF is most often due to the absence of sperm penetration but the reason why fertilization does not occur when intracytoplasmic sperm injection (ICSI) is applied is not clear. However some intrinsic oocyte problems may also be responsible for fertilization failure. It has been reported that 13% of unfertilized oocytes after IVF show morphological abnormalities. When cumulus cells were denuded for ICSI, 60-70% of all oocytes retrieved showed abnormal morphological characteristics.

**Materials and Methods:** Data collection was done using the internet to search Medline for obtaining evidence-based medicine reviews. Cross-references were obtained from key articles. Websites of government bodies and medical associations were searched for guidelines relating to Role of oocyte morphology on ART outcome.

**Results:** In women undergoing ICSI for fertilization assessment of OCCC morphology is pointless, since there is no correlation between OCCC morphology and fertilization and cleavage. first polar body morphology assessment may not serve as a reliable marker of oocyte quality and competence. embryo developmental rate and implantation potential is not affected by the Extracytoplasmic abnormalities of the oocyte. only severe cytoplasmic defects such as organelle clustering/centrally located granulation, appearance of SER clusters, and certain types of fluid-filled vacuoles should be considered as abnormalities.

**Conclusion:** It seems morphological assessment of oocyte is necessary as a predictor for ART outcome. But effects of some abnormalities on fertilization and embryo development are controversial.

**Keywords:** Oocyte Morphology, ART Outcome

### **P-26: The Effects of Interferon-Gamma on Carbamazepine-Induced Fetal Malformations in Mouse Fetus**

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**Background:** One in every 250 newborns is exposed to antiepileptic drugs, in utero. The risk of congenital malformations in newborns, prenatally exposed to antiepileptic drugs (AEDs), is around 5% which is 2 to 2.5 times that of general population; while carbamazepine (CBZ) and valproate (VPA) still provide the treatment of choice in most forms of epilepsy, being efficacious in approximately two-thirds of all newly referred patients. For

unknown reason, nonspecific stimulation of the maternal immune system in pregnant mice has shown what to be a broad-spectrum efficacy for reducing birth defects. The aim of this study is to look for the relationship between maternal immune stimulation and probable reduction of CBZ-induced congenital defects in mouse fetus.

**Materials and Methods:** In order to get this job done, pregnant mice are divided to 5 groups with 20 pregnant mice in each. Finally in the 18th day of pregnancy, after weighting the pregnant mouse, the mouse is killed and after observing the uterus position and the fetuses the following organs of the fetuses are taken out for further histological study: the kidney, liver, thymus, thyroid, central nervous system, heart, and the skeleton of their limbs. Also the liver and kidney of the mother mouse is removed for more study. The result data will be studied by the SPSS software, by a statistician.

**Results:** So far 1st, 2nd, and the 4th groups are studied for the uterus position, and the number of fetuses and their general appearance. In the 4th group the average number of absorbed fetuses was more than the first two groups while the mean weight of fetuses of 4th group was lower. The fetuses of this group also had more creases on their body, some of them also had malformations.

**Conclusion:** It seems that maternal immune stimulation of the pregnant mouse with Interferon-gamma reduces the risk of fetal malformations in mouse fetus.

**Keywords:** Carbamazepine, Fetal Malformation, Interferon-Gamma

### **P-27: The Effect of Oral Morphine Consumption on Plasma Corticosteron Density and Placenta Development in Pregnant Wistar Rats**

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**Background:** Previous studies have shown that morphine consumption during pregnancy may delay embryo development or cause abnormal nervous system function. The most attention of research focuses on the embryo, whereas it was not attended to the placenta as an important organ which is affected by opioids. The present study focused on the effect of maternal morphine consumption on development of maternal portion and fetal portion of placental in Wistar rats.

**Materials and Methods:** In this research, we used 24 Wistar rats with 170-200g weight. The experimental groups after pregnancy received 0/05mg/ml of morphine by tap water while the control group received water. Treatment and control groups were anesthetized by chloroform on 10th and 14th day of pregnancy, placenta with uterus were removed surgically and fixed in 10% formaldehyde for 20 days. 1cc blood was received from pregnant mother, the density of blood corticosteron was determined by ELISA method after centrifugation. The fixed embryos underwent tissue processing, sectioning and staining with hematoxylin and eosin(H&E). Placenta was studied in the light of the thickness of layer, area of blood lacuna, number of maternal and fetal portion cells and thickness of endometrium by light microscope, SPSS analyses and Motic software.

**Results:** Our studies indicated that plasma corticosteron in the treatment group showed severe increase than the control group. Similar result repeated for the experimental group showing the thickness of maternal portions and fetal portions of placenta in day 10 -14 of pregnancy, show the significant difference in experimental group ( $p \leq 0.05$ ). Moreover, the increase number of both maternal and fetal portion cells of placenta and the decrease of blood lacuna area in both fetal and maternal portion Placenta were found, in the treatment group.

**Conclusion:** In this study, the effects of morphine in increase the density of blood plasma corticoestron in addictive pregnant mothers were seen. also, all development of placenta in the experimental group with was delayed related to development in the control group. This may cause embryo abortion and defect of secretory function in placental hormones such as strogen and progesteron which in turn may induce defect in infants development born from addictive mothers.

**Keywords:** Fetal Portion Placenta, Maternal Portion Placenta, Blood Lacuna, Morphine, Rat

### **P-28: Hydrostatic Pressure Induced Cell Death in Cumulus Cells and Improved *In vitro* Maturation of Oocytes from Preovulatory Follicles**

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**Background:** Cryopreservation of ovaries is an important technique in assisted reproduction technology. Physical forces like hydrostatic pressure have a pivotal role in reproduction systems. Due to changes in intra-follicular pressure during ovulatory process, this study designed to examine the effects of hydrostatic pressure on oocyte maturation and cell death in cumulus cells from cryopreserved ovary.

**Materials and Methods:** Ovaries obtained from matured balb/c mice and cryopreserved in ethylene glycol

and DMSO in MEM- $\alpha$  with FBS in straw. Preovulatory follicles isolated from frozen/thawed and fresh ovarian tissues, divided into pressure positive (PP) and pressure negative (PN) groups. In PP groups, follicles were subjected to 20 mmHg hydrostatic pressures for 30 minutes. Follicles were cultured in maturation medium for 24h and then assessed for in vitro maturation of oocyte. Viability of cumulus cells and oocyte were assessed with nuclear differential staining. TUNEL assay was used to detect apoptosis in cumulus cells.

**Results:** Oocyte maturation and cell death in cumulus cells were increased in PP groups and viability of cumulus cells was decreased significantly. Hydrostatic pressure also didn't changed viability of oocyte in all groups. **Conclusion:** Hydrostatic pressure had the mild effect on cell death incidence in cumulus cells without negative effect on oocyte viability. It can be used to improve oocyte maturation in fresh and frozen ovaries.

**Keywords:** Apoptosis, Hydrostatic Pressure, Cumulus Cells, Cryopreservation, Mouse

### **P-29: Effects of Growth Factors and Granulosa Cell Coculture on *In vitro* Maturation of Oocytes**

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**Background:** The maturation medium for in-vitro oocyte maturation is usually supplemented with serum. However, supplementation of serum from pregnant women adversely affects the outcome of in-vitro maturation. The purpose of the study was to assess if growth factors or granulosa cell coculture could overcome the adverse effects of pregnant women's serum.

**Materials and Methods:** The basal maturation medium consisted of TCM 199, 75 mIU/ml hMG, 0.2 mM pyruvate, and 10% serum. The maturation medium for control-1 contained fertile women's serum. The maturation medium for control-2 contained pregnant women's serum. The maturation media for the study groups consisted of medium for control-2, with the addition of EGF, IGF-1, activin, TGF- $\beta$ , or granulosa cell coculture. Immature oocytes were obtained from FVB mice, and the experiment was repeated 6 times. After maturation, the oocytes were fertilized and cultured to blastocysts, and the cumulus cells were analyzed for apoptosis.

**Results:** The maturation, fertilization, and blastocyte rates of control-2 group were significantly lower than

those of control-1 group. Addition of EGF, IGF-1, activin, TGF- $\beta$ , or granulosa cell coculture could not improve the outcome of in-vitro maturation. Cumulus cell proliferation was inhibited by pregnant women's serum. Apoptosis of cumulus cell was not correlated with in-vitro oocyte maturation and subsequent embryo development.

**Conclusion:** The pregnant women's serum has detrimental effects on in-vitro maturation of oocytes, and the addition of growth factors or granulosa cells could not overcome its detrimental effects. The detrimental effects may act via inhibition of cumulus cell proliferation.

**Keywords:** *In vitro* Maturation, EGF, IGF-1, Activin, TGF- $\beta$ , Granulosa Cell, Coculture, Apoptosis

### **P-30: Studies on Morphological Features of Foetal and Adult Ovaries in Kano Brown Goats**

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**Background:** To study the morphological development and histomorphometry of foetal and adult ovaries in Kano brown goats.

**Materials and Methods:** Fifty-one foetal and fourteen adult ovaries obtained from slaughtered Kano brown does in Nsukka abattoir were used for this study. The ages of the adult does were determined by dentition and foetuses by crown rump length method. The foetal and adult ovaries were divided into five different groups using specific age intervals as Gestation day (GD) 50 - 65, 66 - 95, 96 -125 and 126 - 145 and adults. For histological studies the ovaries were fixed, processed and routinely stained with H & E. The ovarian follicles were classified into 5 types according to granulosa cell layers surrounding the oocytes. The number of ovarian follicles per microscopic field, number of granulosa cells surrounding type 1 and 1A follicles and diameter of the ovarian follicles were determined for each group at X400 magnification.

**Results:** The adult mean ovarian weights were significantly higher ( $p < 0.01$ ) than those of the foetuses. Microscopically, the GD 50 - 65 ovaries had no distinct cortex and medulla. Oogonia were numerous among other stromal cells toward the periphery of the ovary. By GD 66 - 95 the ovaries contained types 1, 1a, 2 and 3 follicles. GD 96 - 125 ovaries contained type 4 follicles with early antrum formation and those of GD 126 - 145 comprised type 5 among other follicles. The adult ovaries comprised all the ovarian follicle types. The number of type 1 follicles increased significantly ( $p < 0.01$ ) with foetal age. It was least in the adults. The diameter of adult follicles was significantly higher ( $p < 0.01$ ) than those of the foetuses.

**Conclusion:** The GD 50 - 65 ovaries comprised many oogonia and no distinct cortex and medulla in Kano

brown goats. By GD 66 – 95 they comprised distinct cortex, medulla, types 1, 1A, 2 and 3 follicles. In addition, GD 96 – 125 and 126 – 145 ovaries contained types 4 and 5 follicles respectively. The mean number of type 1 follicles increased significantly with the foetal age while it was least in the adults.

**Keywords:** Adults, Foetuses, Goats, Morphology, Ovaries

### **P-31: Effect of Cryotop Vitrification on DNA Methylation Pattern of Oct4 and Mest Genes in Murine Preimplantation Embryos**

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**Background:** Because of the protection of surplus embryos, Cryopreservation is usually used in ART. It is not clear, the vitrified-warmed embryos that have normal morphology, be normal in genetic level, too. DNA methylation of gene regulatory regions can causes inhibition of gene expression. We study effect of vitrification method of cryopreservation on DNA methylation and gene expression level in investigated genes.

**Materials and Methods:** Two-cell embryos of superovulated mouse were collected by oviduct flushing method, then were divided in control and vitrified-warmed groups. In control group, these embryos were cultured to blastocysts and in other group, they were cultured to 4-8 cell embryos, then were vitrified with CryoTop and after 2-6 months, they thawed and cultured to blastocysts. DNA and RNA of morphologically normal embryos were purified. For investigation of DNA methylation status and quantitative expression of these genes were used Bisulfite-Sequencing Technology and Real time RT-PCR, respectively.

**Results:** The results of quantitative PCR analysis showed that expression level of both genes, Oct4 and Mest, in vitrified-warmed group relative to control group has reduced, also investigation of DNA methylation appeared that in vitrified-warmed group, imprinting status of Mest gene has disturbed and in this group, one CpG dinucleotide in promoter of Oct4 gene has been hypermethylated.

**Conclusion:** This study revealed that CryoTop vitrification has a negative effect on expression level of Oct4 and Mest genes and it seems that this vitrified method can change DNA methylation pattern of these two genes. Presumably, reduction of expression level of Oct4 gene is related to hypermethylation of mentioned CpG dinucleotide in this gene.

**Keywords:** Vitrification, CryoTop, DNA Methylation, Oct4 Gene, Mest Gene

### **P-32: Cumulus Cell Features and Nuclear Chromatin Configuration of *In vitro* Matured Canine COCs and the Influence of *In vivo* Serum Progesterone Concentrations of Ovary Donors**

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**Background:** The objectives of this study were: a) to observe the influence of cumulus investment expansion on the nuclear chromatin configuration of canine oocytes matured in vitro; b) to examine the relationship between cumulus cell (CC) expansion and its morphology after in vitro maturation (IVM); c) to ascertain the influence of in vivo serum progesterone (SP) concentrations of ovary donors on oocyte nuclear maturation, CC phenotypes and degrees of CC expansion of in vitro matured COCs.

**Materials and Methods:** After 48 h of IVM in modified TCM 199, CCs from grade 1 and 2 COCs were stained with propidium iodide. Oocyte chromatin configuration was visualized by Hoechst 33342 stain.

**Results:** The results showed that oocyte IVM was not influenced by degree of CC expansion (D1, D2, and D3) in COCs. From the CC types (C1, C2, and C3), number of C1 types was higher at D1 expansion and differed from those observed at D2 and D3 expansions. Additionally, rates of apoptosis in D1 CCs were lower than those observed in D2 CCs ( $p < 0.05$ ). Oocyte nuclear maturation was not influenced by in vivo SP concentrations of ovary donors. On the other hand, D3 expansion prevailed in COCs from bitches at  $SP > 2.5$  ng/ml ( $p < 0.001$ ). Moreover, in vitro CC apoptosis was associated both with low ( $< 1$  ng/ml) and with high ( $> 5$  ng/ml) in vivo SP levels.

**Conclusion:** These findings indicate that morphology of CCs from in vitro matured dog oocytes gives valuable information on viability of COCs and could possibly be used as a parameter in predicting the quality of oocytes destined for in vitro fertilization (IVF) and their outcomes.

**Keywords:** Dog, Oocyte, Cumulus Cells, Apoptosis, Progesterone

### **P-33: The Effect of Vitrification on Integrity of Zona Pellucida and Blastocyst Surveillance**

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**Background:** In spite of the great advances in vitrification, the mechanism of embryo damage which occurs in some cases and varies from one case to another case is not exactly understood. Since Zona pellucida has an important role on early embryonic development, the aim of the present study is to investigate the effect of vitrification on integrity of zona pellucida and surveillance of blastocysts in mice.

**Materials and Methods:** For this purpose 30 balb-c mice superovulated and blastocysts were obtained by flushing and divided into control and experimental groups. In control group the blastocysts were transferred to DMEM media. 24 hours after the culture, morphological characteristics of zona pellucid and blastomeres were studied by inverted microscope. In the experimental group, the blastocysts after collection were vitrified for one month. After thawing, the blastocysts were transferred to DMEM media and treated as in control group and compared with each other.

**Results:** The result showed that all of the blastocysts in control group (100%) had intact Zona pellucid and blastomeres appeared normal which were considered as live blastocysts. However, in experimental group, in 40% of blastocysts the Zona pellucid were broken or separated from the blastocysts and blastomeres appeared shrunken and darkened which were considered as non alive blastocysts.

**Conclusion:** Blastocysts damage during vitrification is accompanied with zona breakdown, and it could be concluded that vitrification induced embryonic damages is partly due to the zona breakdown.

**Keywords:** Blastocysts, Vitrification, Zona Pellucida

### **P-34: Effect of Including Growth Factors and Antioxidants in Maturation Medium Used for *In vitro* Culture of Buffalo Oocytes Recovered *In vivo***

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**Background:** This study examined the effect of including one of two growth factors (100 ng/ml IGF-1 or 20 ng/ml EGF) in combination with one of two antioxidants (50 µM cysteamine or 50 µM beta-mercaptoethanol) in *in vitro* maturation of buffalo oocytes, followed by fertilization and subsequent development of embryos.

**Materials and Methods:** The oocytes were recovered by *in vivo* ovum pick-up technique from six Murrah buffalo heifers twice a week over a period of 16 weeks. Immediately after ovum pick-up, oocytes recovered from six donors were allocated randomly to five different maturation treatments. The control treatment was the basic maturation medium (MM; TCM-199 supplemented with 10% FBS, 10 IU/ml LH, 0.5 µg/ml FSH, 1 µg/ml estradiol-17beta and 50 µg/ml gentamicin). The other four treat-

ments consisted of the control maturation medium (MM) plus one combination of a growth factor and an antioxidant viz. IGF-1 + cysteamine; IGF-1 + beta-ME; EGF + cysteamine or EGF + beta-ME. The total number of oocytes assigned to each maturation treatment ranged from 31 to 66. After maturation in different maturation medium, media used for *in vitro* fertilization was same for all groups i.e. mSOF along with some supplements. Following *in vitro* fertilization, the presumptive zygotes were stripped of the cumulus cells and were cultured for subsequent development over cumulus cells monolayer along with active motile bovine oviductal epithelial cells in supplemented mSOF. Data were analysed using Chi-square test.

**Results:** The maturation rate observed for the growth factor plus antioxidant treatments was similar to that for the control (90.4%). The highest cleavage rate recorded in the IGF-1 + cysteamine treatment (71.9%) which was significantly higher ( $p < 0.05$ ) than the IGF-1 + beta-ME (45.2%) and EGF + beta-ME (46.4%) treatments, but not significantly differ from the control (63.8%) and EGF + cysteamine treatment (60.7%). The proportion of cleaved oocytes those developed to blastocyst stage was significantly higher in the IGF-1 + cysteamine treatment (52.2%;  $p < 0.05$ ) than in the control (23.3%), the EGF + cysteamine (13.5%) or the EGF + beta-ME (7.7%) treatments, but did not differ significantly from the IGF-1 + beta-ME (28.6%) treatment. Following non-surgical transfer of 15 embryos to 14 synchronized recipients, four became pregnant and only one recipient sustained the pregnancy as long as 4.5 months when spontaneous abortion occurred. Later by using the combination of IGF-1 (100 ng/ml) + cysteamine (50 µM) and following transfer of 14 embryos their team was first to produce the first buffalo calf through this technology which was named as Saubhagya by the honorable Shri Sharad Pawar, Union Minister of Agriculture of India.

**Conclusion:** It was concluded that supplementing the maturation medium with IGF-1 + cysteamine improved the production of buffalo embryos significantly *in vitro* culture. Also, the technique could be used for rapid multiplication of elite buffalo germ plasm.

**Keywords:** Buffalo, Ovum Pick-Up, Growth Factors, Antioxidants, *In vitro* Embryo Production

### **P-35: Vitrification Induced Apoptosis in Mice Blastocysts**

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**Background:** Apoptosis is induced by several factors and has an important role in embryonic development. Since vitrification-induced embryonic damages could be resulted from an increase in apoptosis occurrence,

the aim of the present study is to investigate the effect of vitrification on induction of apoptosis in blastocysts.

**Materials and Methods:** For this purpose 20 balb-c mice superovulated and blastocysts were obtained by flushing and divided into control and experimental groups. In control group the blastocysts were transferred to DMEM media. 24 hours after the culture, blastocysts were stained with TUNEL technique for apoptosis and propodium iodide for necrosis. The stained blastocysts were studied with fluorescent microscope. In the experimental group, the blastocysts after collection were vitrified and kept in liquid nitrogen for one month. After thawing, the blastocysts were treated as in control group and then compared with each other.

**Results:** According to the method of study the apoptotic cells had a green fluorescence and nonapoptotic cells had red fluorescence. The result showed the apoptotic index in control and experimental group was  $8.72 \pm 57$  vs  $10.72 \pm 87$  respectively. There was not a significant difference regarding the number of apoptotic cells in control and experimental group.

**Conclusion:** According to the results it appears that vitrification - induced embryonic damages is not mediated by apoptosis.

**Keywords:** Blastocysts, Vitrification, Apoptosis

### **P-36: Crocin Improves Maturation Rate of Immature Mouse Oocytes**

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**Background:** The pregnancy rates from oocytes matured in vitro are much lower than those of in vivo stimulation cycles, indicating that optimization of IVM remains a challenge. Reduced developmental competence of oocytes matured in vitro indicates that current culture systems for oocytes maturation do not adequately support nuclear and/or cytoplasmic maturation. Therefore in this study, we evaluated the effect of different concentrations of crocin as an antioxidant agent on in vitro maturation (IVM) of immature mouse oocytes (COCs).

**Materials and Methods:** Cumulus-Oocytes Complex (COCs) were collected from 6-8 weeks old NMRI female mice. Cocs were cultured in IVM medium supplemented with 0 (control), 50, 100, 200 and 400 µg/ml crocin for 16-18 h in 5% CO<sub>2</sub> and 37°C. Then the rates of maturation were recorded.

**Results:** Our data showed that addition of 50 µg/ml crocin to maturation medium significantly ( $p < 0.05$ ) increased in vitro maturation of oocytes in compare with control group ( $70.3 \pm 2.4$  vs  $56 \pm 3.9$ ).

**Conclusion:** These results suggested that crocin could increase oocyte maturation rate. Therefore, addition of crocin as an antioxidant can improve oocyte maturation.

**Keywords:** Oocyte, *In vitro* Maturation, Cocin, Antioxidant

### **P-37: The Ejaculatory Duct Ectopically Invading Towards the Bladder with Multiple Congenital Malformations of the Homolateral Urogenital System: A Report of a Rare Case and an Embryological Review**

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**Background:** To report a rare case of a left ejaculatory duct that allotropically protrudes or invades towards the left vesicle triangular area with its dead end.

**Materials and Methods:** The patient simultaneously exhibited multiple congenital malformations of the homolateral urogenital system, such as the absence of a left kidney, the dysplasia and allotopia of the left seminal vesicle, the absence of the left ureterostoma, separation between the left testis and the epididymis tail, and the maldevelopment of the left testis.

**Results:** According to all clinical and laboratory evidence, the case represented a new syndrome, which we named Wuyang's syndrome.

**Conclusion:** It involved a rare phenomenon in embryonic development: the dysplastic proximal vas precursor (PVP), having intruded into a common mesonephric duct (CMD) and accidentally encroaching on the ureteric bud (UB) position, resulted in the absence or dysplasia of the homolateral urinary tract and ectopic invasion of the bladder by the homolateral seminal tract.

**Keywords:** Common Mesonephric Duct, Proximal Vas Precursor, Ureteric Bud, Urogenital System

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## **Epidemiology and Ethics**

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### **P-38: Lipid Profile in Women with Polycystic Ovary Syndrome (PCOS): A Comparative Study**

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**Background:** Polycystic ovary syndrome (PCOS) is a

disorder of hyperandrogenemia and chronic anovulation which affects 5-10% of all women. It has been reported that women with PCOS often have serum lipid level elevation. This study compares serum lipid levels in women with and without polycystic ovary syndrome.

**Materials and Methods:** In this comparative-cross sectional study, lipid profiles (Total cholesterol, Apo lipoprotein A-I, Apo lipoprotein B, HDL, LDL, TG, and lipoprotein a) in 154 women (57 with and 96 without PCOS) were compared.

**Results:** There were no differences between two groups in terms of age, age of menarche and body mass index (BMI). Serum levels of total cholesterol, Apo lipoprotein A-I, Apo lipoprotein B, HDL, LDL, TG, and lipoprotein a didn't show any differences between two groups, but Apo Lipoprotein A-1 level was higher in women with PCOS in comparison with no PCOS. When subjects were classified according to their BMI, similar results were obtained for those with BMI less than 25. However, PCOS subjects with BMI higher than 25 had a significantly higher serum level of total cholesterol, Apo lipoprotein A-I, LDL, and TG than PCOS subjects with BMI less than 25.

**Conclusion:** The present study doesn't support the notion that PCOS affects serum lipid levels except for Apo Lipoprotein A-1. Future studies are to consider insulin profile in similar comparison

**Keywords:** PCOS, Lipids, Lipid Subgroups, Insulin Resistance

### **P-39: HSV-2 Seroepidemiology and Risk Factors among Iranian Women: A Time to New Thinking**

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**Background:** This study designed to determine and compare the prevalence of HSV-2 infection between two groups of women with high and low risk behaviors in Tehran.

**Materials and Methods:** 362 women attending OB & GY clinics as low risk group and 156 prisoner and DIC (Drop in Center) resident women in Tehran as high risk group, were enrolled in this cross sectional study. HSV-2 antibodies were tested by Enzyme-Linked Immunosorbent Assay (ELISA), Data analyzed by independent T-test, Chi square, Pearson correlation coefficient, uni-

variate and multiple logistic regression model by SPSS software. P-values less than 0.05 were considered as significant.

**Results:** The prevalence of IgG antibody in high risk group was significantly more than low risk women (26.3% vs. 2.5%;  $p < 0.001$ ). The prevalence of IgM antibody in high risk group was less than low risk group (3.8% vs. 7.1%) but the difference was not statistically significant. In high risk group, there was significant association between positive IgG test results and anal/oral sex ( $p < 0.01$ ), condom use ( $p < 0.01$ ), smoking ( $p < 0.05$ ), drug addiction ( $p < 0.001$ ).

**Conclusion:** Relatively high prevalence of genital herpes among high risk women necessitates regular screening and safe sex education programs to prevent the disease transmission to others in the community. Moreover, regard to prevalence rate of 7.1% for IgM antibody in low risk women, risk of acute infection in this group should not be ignored and its distribution in Iranian population should be alarmingly concerned

**Keywords:** Genital Herpes, Women, Behavior, ELISA

### **P-40: "I Want to Bring God in": Religious Women's Experiences and Preferences in Relation to Infertility Counseling**

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**Background:** Religion and spirituality are fundamental parts of culture which influence how individuals experience and interpret infertility counseling. A competent infertility counselor needs a set of culturally sensitive proficiencies regarding religion and spirituality when dealing with infertile women affiliated to diverse religious groups. This study explored Muslim and Christian women's experiences and preferences in relation to infertility counseling.

**Materials and Methods:** Using a grounded theory approach 30 infertile women affiliated to different denominations of Christianity (Protestantism, Catholicism, Orthodoxies) and Islam (Shiite and Sunni) were interviewed. Data were collected through semi structured in-depth interviews in two UK and one Iranian fertility clinics and analyzed using Straussian mode of grounded theory.

**Results:** Religious infertile women experienced infertility as a God-granted phenomenon, God's test and as an enriching experience for spiritual growth. This perspective helped them to get a feeling of self-confidence and empowerment and consequently being able to manage their emotions. Hence, they relied more on their religious coping strategies and less on formal support resources like counseling services. However, they

wished counselors to have an openness and willingness towards taking time to understand and discuss the religious and spiritual concerns in their counseling.

**Conclusion:** We argue that infertility counselors in addition to focusing on the psychosocial needs of infertile women should also consider the religious and spiritual concerns of the clients. Establishing a sympathetic, nonjudgmental and accepting relationship with infertile women will allow them to discuss their religious and spiritual concerns, which consequently enhance their usage of counseling services.

**Keywords:** Infertility, Religion, Spirituality, Counseling, Grounded Theory

### **P-41: Low Birth Weight Prevalence among the Newborns and Potential Risk Factors**

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**Background:** Low birth weight can contribute to many complications in neonatal period and even infancy and later. This study was performed to assess the prevalence of low birth weight in two cities of Iran and also to evaluate the potential risk factors of it.

**Materials and Methods:** This was a cross-sectional study, performed on 1513 pregnant women attending delivery facilities in educational hospitals of Tehran and Noor City (in north of Iran). After obtaining the consent of subjects to enter the study, a general and demographic questioner was completed by the researchers, and all related factors in reproductive and pregnancy history were registered. After the delivery, the neonate's weight was registered and the prevalence of low birth weight in all these deliveries were assessed. Low birth weight was defined as neonatal weight < 2500 grams at birth. Data were entered and analyzed by SPSS-15 and p-values < .05 were considered significant.

**Results:** Of 1513 live newborns in this study, 61(4%) were low birth weight. Potential risk factors of low birth weight including: age of mother, total number of pregnancies, Body Mass Index(BMI) in first prenatal visit- which reflects BMI before pregnancy, previous low birth weight newborn, car and house possession- as an indicator of socio-economic status, active or passive smoking during pregnancy, preterm delivery, consumption of Ferrous Sulfate, Folic acid, multivitamins and calcium during pregnancy, and consumption of Folic Acid from 3 months before pregnancy, were entered in Binary Logistic Regression model and their effects on low birth weight were evaluated. Of all the mentioned factors, only preterm

delivery (OR: 72.94, 95%CI: 37.23-142.91), BMI in first prenatal visit (OR: .92, 95%CI: .84-.99), and Folic Acid consumption from 3 months before pregnancy (OR: .44, 95%CI: .21-.94) had statistically important relationships with low birth weight.

**Conclusion:** Preterm delivery and mother's BMI before pregnancy have been nearly proved to affect the weight of newborns, and are confirmed in this study, but the negative effect of Folic Acid consumption from 3 months before pregnancy on low birth weight is new to us and we recommend that more research on different samples of all parts of Iran be performed, so that the true effects of potential risk factors of low birth weight are known.

**Keywords:** Pregnancy, Low Birth Weight, Risk Factor

### **P-42: Who Owns the Frozen Embryos?**

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**Background:** Success in assisted reproductive techniques is raising hope for many infertile couples, but as in these techniques many embryos are made, there is no choice other than cryopreservation of the not-transferred embryos. Along with development of these techniques, the number of In-vitro made embryos is rising and the number of transferred embryos is decreasing to prevent multiple gestations both leading to increasing the number of spare embryos which must be kept frozen in the IVF centers. Although it has been reported that cryostorage duration do not adversely affect post-thaw survival or pregnancy outcome, the length of time embryos can remain in storage is subject to legislation and differs between countries and even states. Anyway, there is a duration for cryopreservation and so an endpoint for it same as the contracts on cryopreservation of the embryos. It is obvious that at a certain time, owners of frozen embryos must make their decisions about the fate of their embryos, the main problem in Iranian law is: "who is the owner of frozen embryos" the father, the mother or both. This issue is not just limited to the decision about the fate of embryos that can be discarding, donation to other couples or donation for research, there are other situations and problems in which only the owner can make the decision. Situations like divorce, death of one partner, argument between husband and wife, changing the idea or withdrawal of the contract need to exactly clarify who can decide for these embryos and how.

**Materials and Methods:** In this paper we have legal discussion about this issue upon Iran's civil law and Islam concluding that the owner of the embryo is the cou-

ple and both of them must decide for the embryos.

**Results:** Meanwhile, the initial contract is necessary and its content is very important, and also the consent of one part (male or female) to the other one for making the decision cannot be accepted. The demand for use the embryos for the owners also shall be made by both partners.

**Conclusion:** It would be better to give a detail consultation to the couples and mention all the possibilities to prevent any misunderstanding leading to the future arguments.

**Keywords:** Cryopreservation, Legal, Islamic, Embryo, Frozen

### **P-43: Reproductive Toxicity of Low Metal Exposure on Male**

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**Background:** Recent evidence has indicated a deterioration in reproductive health of men in many countries over the past few decades, particularly a decrease in semen quality and an increase in prevalence of prostate cancer. The human male has a relatively low fertility potential as compared to other mammals. Thus, the human male may be at greater risk from reproductive toxicants. For example, the number of sperm per human ejaculate is typically only 2- to 4-fold higher than the number at which fertility is significantly reduced, whereas the number of sperm in rat, rabbit, or bull ejaculate is many times (up to 1400-fold) the number that will produce maximum fertility. The human male may be more susceptible than the rat to metal toxicity, possibly because of the poorer efficacy of the antioxidant defense system [lower systemic levels of glutathione, vitamin C, and glutathione peroxidase, compared to those in the rat]. Furthermore, because of differences among species in reproductive end-points and in the route, level, and duration of metal exposure, experimental animal data cannot be extrapolated to the human situation and may not be useful for estimates of allowable human exposure. The aim of this review study is to explore associations between exposure to metals and male reproductive hormone levels.

**Materials and Methods:** This review article prepared by studying articles obtained from Google, pub med sites with key words such as: Human semen quality; Sex hormones; lead exposure; Toxic and essential metals interaction.

**Results:** An increase in blood lead is associated with increasing in immature sperm concentration, in percentages of pathologic sperm, wide sperm, round sperm, and short sperm, in serum levels of testosterone and estradiol, and a decrease in seminal plasma zinc and in serum prolactin. These reproductive effects were

observed at low-level lead exposure (BPb median 49 mg/L, range 11–149 mg/L) common for general populations worldwide. An increase in blood cadmium was associated with increasing percentage of amorph sperm and serum testosterone. In addition to direct toxicity, Pb and Cd can interfere with the metabolism of certain essential elements including copper (Cu), zinc (Zn) and selenium (Se) by affecting their absorption, distribution, and bioavailability in the body; can contribute to oxidative stress, implicated in the pathogenesis of male infertility; and can inhibit DNA repair. On the other hand, Zn and Se are known to be essential for male reproductive function and can reduce the toxicity of Pb, Cd, and several other metals.

**Conclusion:** The observed significant synergistic effect of blood Pb and blood Cd on increasing serum testosterone, and additive effect of a decrease in serum Se on increasing serum testosterone, may have implications on the initiation and development of prostate cancer because testosterone augments the progress of prostate cancer in its early stages.

**Keywords:** Semen Quality, Sex Hormones, Metal Exposure

### **P-44: Sexual Satisfaction and Marital Satisfaction in 45-65 Years Old Men in North of Tehran**

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**Background:** To determine the relationship between sexual satisfaction and marital satisfaction according to personal characteristics.

**Materials and Methods:** This is Cross sectional study. 200 volunteer healthy 45-65 years old men, who were met in the public centers of well socioeconomic district in North of Tehran, were interviewed. The questioner had two main parts: 1) Personal Characteristics 2) Sexual and marital satisfaction and their changes (0-10 Numerical Scale was used). The descriptive and inferential statistics were used (t test, ANOVA, Scheffe). (2007-2008).

**Results:** There were correlating between sexual satisfaction and marital satisfaction, only significant difference between sexual satisfactions of age group 45-49 (highest satisfaction) with other age groups ( $p < 0.05$ ), and only significant difference between marital satisfaction of age group 45-49 (highest satisfaction) and age 60-65 ( $p < 0.05$ ). We found significant effect of decreasing energy, decrease of sex pleasure... in sexual satisfaction and marital satisfaction ( $p < 0.05$ ). Referring to financial situation since we found highest marital satisfaction and sexual satisfaction in high income, but it was

not significant.

**Conclusion:** We found highest marital satisfaction changes according to sexual satisfaction changes in age 60-65, and lowest one in age 45-49. We suggest more marital and sexual counselling for age over 60. Since most of changes were occurred over age 50, we suggest more education; guiding and counseling program for this group.

**Keywords:** Andropause, Marital Satisfaction, Sexual Satisfaction, Personal Characteristics

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## Female Infertility

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### **P-45: Predictive Value of Follicular Fluid Vitamin D on Assisted Reproductive Technique Outcome**

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**Background:** Vitamin D, a steroid hormone, has critical roles in human health. The available data thus identify vitamin D as an important substance in processes involved in reproductive success and thereby suggest pathophysiological mechanisms for reproductive compromise in the setting of vitamin D deficiency. Realizing the prevalence of vitamin D insufficiency in our country, we hypothesized that deficient vitamin D stores will translate to decreased reproductive success following ART. This study aimed to determine whether 25OH-D levels in the follicular fluid of infertile women undergoing ART demonstrate a relationship with stimulation cycle parameters and outcome.

**Materials and Methods:** A prospective cohort study was undertaken at Infertility department of Shariati Hospital affiliated to Tehran University of Medical Sciences. Eighty-two infertile women undergoing assisted reproductive technique were enrolled between 2009 and 2010. The study protocol was in accordance with the guidelines of Declaration of Helsinki, approved by institutional review board of the Tehran University of Medical Sciences. All patients underwent ART cycles with standardized regimens for COH after long protocol pituitary down regulation. Serum samples of Vitamin D (25OH-D), calcium, phosphorus, alkaline phosphatase, parathormone were collected. Transvaginal ultrasound guided oocyte retrieval was performed 36 hours following the hCG injection. Follicular fluid was collected from follicles  $\geq 14$ mm; following oocyte isolation, follicular fluid for each patient was pooled, centrifuged and the supernatant was stored until assayed for Vitamin D (25OH-D). Fertilization was assessed 24 hours after insemination. Fresh embryo transfer was performed on day 3 after insemination. The luteal phase was supported by intramuscular and vaginal

progesterone. ART cycle parameters were determined. Positive serum hCG tested 14 days after embryo transfer was considered as evidence of implantation. Clinical pregnancy was defined as intrauterine gestational sac visible on transvaginal ultrasound.

**Results:** Baseline characteristics and ovarian stimulation parameters of the under study patients were similar. Based on previously defined serum criteria, serum and follicular 25OH-D level  $>30$ ng/mL was defined to reflect "replete" vitamin D status; level between 20-30 ng/mL was taken to reflect vitamin D insufficiency, whereas 25OH-D level  $<20$ ng/mL defined evidence of vitamin D deficiency. In accordance with previous prevalence studies in our country, vitamin D insufficiency and deficiency are common health problems which require emergent attention of health professionals. Follicular 25OH-D level has positive direct correlation with serum 25OH-D. Pregnancy rates did not differ significantly between tertiles.

**Conclusion:** Our findings revealed that vitamin D deficiency may not have an important role in ART outcomes. The potential for benefit of vitamin D supplementation on treatment success in infertile patients undergoing ART is debated and merits further investigation.

**Keywords:** Vitamin D, 25OH-D, Infertility, Pregnancy, Follicular Fluid

### **P-46: The Effectiveness of Clomiphene Citrate in LH Surge Suppression in Women Undergoing IUI: A Randomized Controlled Trial**

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**Background:** To study the effectiveness of Clomiphene citrate (CC) in preventing a premature LH surge during controlled ovarian stimulation in women undergoing assisted reproduction.

**Materials and Methods:** Couples were randomized to receive human menopausal gonadotrophins (hMG) followed by CC or hMG alone till the day of hCG. The primary outcomes were the incidence of a clinical pregnancy and premature LH rise. Secondary outcomes were the estradiol levels, number of mature follicles, and endometrial thickness, as determined on the day of hCG.

**Results:** The number of patients who had a premature LH surge was significantly less in the hMG + CC group (5.45% vs. 15.89%,  $p < 0.001$ ). Additionally, the mean E2 levels (pg/mL) and the number of mature follicles were also significantly higher in the hMG + CC group [(360.3  $\pm$  162.9 vs. 280  $\pm$  110.0,  $p < 0.05$ ) and (2.4  $\pm$  0.97 vs 1.3  $\pm$  1.1,  $p < 0.05$ ), respectively] albeit, there was no significant difference regarding the number of cancelled cycles, the endometrial thickness or the clinical pregnancy rate.

**Conclusion:** The addition of CC to hMG has been proven effective in reducing premature LH surges without compromising the pregnancy rate. This novel protocol should be tested in minimal IVF stimulation protocol.

**Keywords:** Prevention, LH Surge, Clomiphene Citrate

#### **P-47: Are There any Predictive Factors for Successful Intracytoplasmic Sperm Injection. Statistical Analysis of 339 Cycles**

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**Background:** Intracytoplasmic sperm injection (ICSI) outcome is tightly depending of male and female factors. The assessment of several clinical and laboratory parameters may predict results of ICSI.

**Materials and Methods:** We retrospectively analysed patients who had ICSI for male or female factor infertility. The clinical and laboratory factors that influenced the fertilization, pregnancy and implantation rates were also analysed. 139 cycles in 269 couples were analysed. women's age, etiology of infertility, duration of infertility number of retrieved oocytes, sperm parameters, number of transferred embryos, body mass index, pelvic disease and transfer day were evaluated.

**Results:** Optimal pregnancy rates were observed in women aged 25-35 years, with gradual decline with advanced age. The pregnancy/transfer rate was statically depending of, the number of retrieved oocytes and the number of transferred embryos. However, transferring more than three embryos was no significantly superior to two or three. Etiology of infertility had no influence in fertilization and pregnancy, but may predict the implantation rate. The duration of infertility was of no value in predicting the fertilization, implantation or pregnancy rates, and neither seem's to be the sperm parameters.

**Conclusion:** The only statistically significant variable of ICSI outcome were women partner's age, number of retrieved oocyte and number of transferred embryos.

**Keywords:** Intracytoplasmic Sperm Injection (ICSI)- Male and Female Factors

#### **P-48: Infertility and Stress**

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**Background:** Infertility is one of the major problems in life which affects about 20% of couples in developed countries and a large population in the developing countries including Iran. Since not much has been discussed, published or talked about in the literature and infertili-

ty conferences in Iran, we have decided to conduct a review of literature and provide a summary of what is known in the world regarding this subject.

**Materials and Methods:** We have conducted a thorough literature search using PubMed and other major search engines in the medical databases and collected the data and research results regarding infertility and stress.

**Results:** Infertility is often influenced by psychological factors and the two most commonly investigated psychological problems in infertility are anxiety and depression. In most studies, it is suggested that psychological distress is associated with infertility and infertility treatment. In some recent studies, it has been concluded that stress may be a causal factor that leads to infertility. Preexisting anxiety and/or depression have been reported in the literature to be negatively associated with successful conception. The reciprocal relationship exists between stress and infertility.

**Conclusion:** We suggest that psychological treatments should be considered for infertile couples before infertility treatment is initiated in order to increase the possibility of spontaneous conception in women.

**Keywords:** Infertility, Stress, Human, Women, Psychology

#### **P-49: The Effects of Electro Acupuncture on Body Composition and Infertility in Iranian Obese and Overweight Women**

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**Background:** Obesity is linked to several health conditions, including cardiovascular disease, hypertension, diabetes and infertility. Obesity results in an increased production of estrogen. Women who are overweight or obese are less likely to respond to fertility drugs, because excess weight interferes with the proper absorption of a variety of drugs used in infertility treatment. Electro acupuncture has been reported to reduce body weight, BMI, body and trunk fat mass in subjects in clinical practice.

**Materials and Methods:** In the present study, we have evaluated the effects of Electro acupuncture on body composition including body weight, BMI and body fat mass in women divided into 2 groups as following; Case Group: (n=58) subjects with low-calorie diet and Electro acupuncture. Control group: (n=58) subjects with low-calorie diet and sham Electro acupuncture. Body weight, BMI, body fat mass, trunk fat mass measured

for three times in all subjects, first; at the beginning of study, second; one day after treatment and third; 45 days after treatment.

**Results:** There was a statistically significant reduction in body weight ( $p<0.000$ ), BMI ( $p<0.000$ ), body fat mass ( $p<0.000$ ) pre and post treatment in the case and control groups. With comparison of the reduction in these groups, we saw the significant reduction in case group ( $p<0.05$ ). There was a statistically significant reduction in trunk fat mass ( $p<0.000$ ), arms fat mass ( $p<0.01$ ) and legs fat mass ( $p<0.000$ ) pre and post treatment in both the case and control groups. In comparison of these groups, we know the reduction of case group is not significant in trunk fat mass ( $p>0.1$ ), and arms fat mass ( $p>0.1$ ), but it was significant in legs fat mass ( $p<0.05$ ). These reductions were continued after 45 days in each case and control groups. In subjects with Excess and Deficiency patterns these results were nearly the same ( $p>0.1$ ).

**Conclusion:** We found that the electro acupuncture supplements had a significant effect in body weight, BMI, body fat mass especially legs fat mass and did not have significant effects on arms and trunk fat mass.

**Keywords:** Infertility, Electro Acupuncture, Body Weight, BMI, Body Fat Mass, Trunk Fat Mass, Legs Fat Mass, Arms Fat Mass

### **P-50: Auricular Acupuncture Has Beneficial Effects on Body Composition and Infertility in Iranian Obese and Overweight Women**

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**Background:** Obesity and infertility have been linked together by several studies. Obesity results in an increased production of estrogen; this hormonal imbalance in turn interferes with ovulation, which of course, is the basis of successful conception. Auricular acupuncture is one of the acupuncture therapies used to prevent and treat obesity.

**Materials and Methods:** In this research we used auricular acupuncture for treatment. In this study, women divided into 2 groups as following; Case Group: (n=42) subjects with low-calorie diet and Auricular acupuncture. Control group: (n=42) subjects with low-calorie diet and sham Auricular acupuncture. Body weight, BMI, body fat mass, trunk fat mass measured for three times in all subjects, first; at the beginning of study, second; one day after treatment and third; 45 days after treatment.

**Results:** There was a statistically significant reduction

in body weight ( $p<0.000$ ) and BMI ( $p<0.000$ ), body fat mass ( $p<0.000$ ) pre and post treatment in the case and control groups. With comparison of the reduction in these groups, we saw the significant reduction in case group ( $p<0.001$ ). There was a statistically significant reduction in trunk fat mass ( $p<0.000$ ), arms fat mass ( $p<0.01$ ) and legs fat mass ( $p<0.000$ ) pre and post treatment in both the case and control groups. In comparison of these groups, we know the reduction of case group is not significant in legs fat mass ( $p>0.1$ ), and arms fat mass ( $p>0.1$ ), but it was significant in trunk fat mass ( $p<0.05$ ). These reductions were continued after 45 days in each case and control groups.

**Conclusion:** We found that the Auricular acupuncture supplements had a significant effect in body weight, BMI, body fat mass especially trunk fat mass and did not have significant effects on arms and legs fat mass.

**Keywords:** Infertility, Auricular Acupuncture, Body weight, BMI, Body Fat Mass, Trunk Fat Mass, Legs Fat Mass, Arms Fat Mass

### **P-51: The Role of Metformin in Reduction Serum C-Reactive Protein Levels in Women with Polycystic Ovary Syndrome**

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**Background:** Low-grade chronic inflammation, reflected in elevated levels of serum C-reactive protein (CRP), has recently been linked to obesity, insulin resistance syndromes such as polycystic ovary syndrome (PCOS), and an increased risk of cardiovascular disease. Because the insulin sensitizer metformin has been shown to improve metabolic disturbances in PCOS, it was of particular interest to examine serum CRP levels during metformin therapy.

**Materials and Methods:** Sixty women with PCOS were randomized to receive either metformin (500 mg twice daily for 6 months, N=30) or oral contraceptive pills (for 6 months, N=30). Serum concentrations of CRP was measured at the start and the end of study in both groups.

**Results:** The serum concentrations of CRP were significantly higher in PCOs women. In metformin group treatment, serum CRP levels decreased significantly from  $3.08 \pm 0.7$  mg/liter to  $1.52 \pm 0.26$  mg/liter at 6 months ( $p=0.006$ ). In contrast, oral contraceptive pills group increased serum CRP levels from  $2.91 \pm 0.68$  mg/liter to  $4.58 \pm 0.84$  mg/liter ( $p<0.001$ ). Whether this effect is related to estrogen action in the liver or whether it reflects increased inflammation process and possible risks for cardiovascular disease remains unclear.

**Conclusion:** The decrease of serum CRP levels during metformin therapy is in accordance with the known ben-

eficial metabolic effects of this drug and suggests that CRP or other inflammation parameters could be used as markers of treatment efficiency in women with PCOS.

**Keywords:** Polycystic Ovary Syndrome, C-Reactive Protein, Metformin

### **P-52: Controlled Ovarian Hyperstimulation and Intrauterine Insemination Cycles in Patients with Unilateral Tubal Blockage Diagnosed by Hysterosalpingography**

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**Background:** Tubal pathology ranks among the most frequent causes of subfertility, next to ovulatory disorders and sperm defects. Therefore, assessment of tubal patency is a fundamental part of infertility workup. Investigation for tubal disease can be divided into radiological tests: hysterosalpingography (HSG), selective salpingography and hystero-contrast-sonography (HyCoSy), microbiological tests: Chlamydia testing of the serum or urine and surgical tests: laparoscopy with chromoperturbation, falloscopy; fertiloscopy. Although, diagnostic laparoscopy is generally accepted as the most accurate procedure to detect tubal pathology and periadnexal adhesions and endometriosis owing to the noninvasive nature and low cost, HSG is widely used as a first-line approach to assess uterine anatomy and tubal patency in routine infertility workup. HSG has 65% sensitivity and 83% specificity for tubal obstruction. A good correlation between HSG and laparoscopy regarding tubal patency has demonstrated. There is a growing tendency to pass diagnostic laparoscopy in couples with a normal HSG (patent tubes) who will undergo intrauterine insemination (IUI) treatment for unexplained infertility (UEI), mild male subfertility and cervical hostility. Management of the patients with unilateral tubal blockage diagnosed by HSG is controversial subject. Although, a number of reports recommended laparoscopy and dye test to confirm or refute the diagnosis, then reconstructive tubal surgery by laparoscopy, selective salpingography and tubal catheterization (SS/TC) or hysteroscopic transcervical tubal cannulation. The other practitioners suggested that one-sided tubal pathology does not influence the possibility of treatment independent pregnancy. and suggested that laparoscopy may be omitted in women with normal HSG or suspected unilateral tubal pathology on HSG, since it was not shown to change the original treatment plan indicated by HSG in 95% of the patients, and showed that bilateral tubal pathology diagnosed at HSG or laparoscopy did affect fertility prospects strongly, whereas unilateral pathology affected future fertility less severely, and recommended controlled ovarian hyperstimulation (COH) and IUI as the initial treatment of choice in patients with unilater-

al tubal occlusion diagnosed by HSG. So far, only one retrospective study with relative small sample size has tried to evaluate pregnancy rates after COH and IUI in women with HSG findings suspicious to unilateral tubal occlusion. Thus we decided to carry out a prospective study to assess the therapeutic value of COH and IUI in these patients.

**Materials and Methods:** Cross-sectional analysis, between October 2006 and October 2009. In an Academic reproductive endocrinology and infertility center, Mirza Koochak Khan. Two groups of patients undergoing stimulated IUI cycles were compared. Sixty-four infertile couples with unilateral tubal blockage diagnosed by HSG as the sole cause of infertility in the study group, and two hundred couples with unexplained infertility in the control group. The patients underwent 3 consecutive ovarian hyperstimulation (Cl miphen citrate and human Menopausal Gonadotropin) and IUI cycles.

**Results:** Demographic data were found to be homogenous between the study and control groups. Cumulative pregnancy rates were similar in the study group (26.6%) and the control group (28%) ( $p=0.823$ ;  $OR=1.075$ ; 95% CI: 0.57-2.28). The cumulative pregnancy rate in subgroup with mid-distal tubal blockage (16%) was not statistically lower than subgroup with proximal tubal blockage (33%) ( $p=0.15$ ;  $OR=2.625$ ; 95% CI=0.745-9.25). The cumulative pregnancy rate in subgroup with mid-distal tubal blockage (16%) was lower than the patients with unexplained infertility (28%), the difference was not statistically significant ( $p=0.209$ ;  $OR=2.042$ ; 95% CI=0.671-6.213).

**Conclusion:** Controlled ovarian hyperstimulation and IUI could be recommended as the initial treatment in sub-fertile couples with unilateral tubal blockage as the sole cause of infertility.

**Keywords:** Hysterosalpingography, Intrauterine Insemination, Ovarian Stimulation, Tubal Factor Infertility, Unilateral Tubal Blockage

### **P-53: The Unexpected Occurrence of Spontaneous Pregnancy during Hormone Replacement Therapy for Premature Ovarian Failure**

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**Background:** Premature ovarian failure (POF) is defined by the association of amenorrhea, hypogonadism and elevated (menopausal) levels of serum gonadotropins before age of 40 years-old. This condition affects approximately 1% of women under 40 years of age. Physiologic replacement of ovarian steroid hormones seems rational until the age of normal menopause. Temporary return of ovarian function, as indicated by elevated estradiol levels, and follicle development may occur in this condition. Spontaneous Pregnancy is a very rare event in the patients with POF. The choice

therapeutic protocol for infertility treatment is IVF program with egg donation. We report a case of POF who conceived spontaneously during hormone replacement therapy.

**Materials and Methods:** An infertile 30 years-old woman was presented with confirmed premature ovarian failure after pelvic surgery ( Serum FSH levels in range of 62-135 IU/ L, LH levels in range of 34.8-88IU/ L ,Progesterone level 0.3ng/m L, and those of estradiol less than 10pg/ m L) on hormone replacement therapy, who conceived. She is currently in second trimester (23weeks) of an uneventful pregnancy.

**Results:** Despite having ovarian failure and receiving hormone replacement therapy, the patient spontaneously conceived.

**Conclusion:** Return of ovarian function and achievement of pregnancy is possible in infertile women with premature ovarian failure. Women who wish to avoid pregnancy should use proper contraceptive methods.

**Keywords:** Premature Menopause, Premature Ovarian Failure, Transient Ovarian Failure, Hypergonadotropic Hypogonadism, Hypergonadotropic Amenorrhea, Hormone Replacement

#### **P-54: The Effect of Luteal Phase Support on Pregnancy Rate of Stimulated IUI Cycles in Unexplained Infertility**

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**Background:** Progesterone (P) that is produced by the corpus luteum in response to stimulation by luteinizing hormone (LH) and human Chorionic Gonadotropin (hCG) in luteal phase is essential for secretory transformation of endometrium that permits implantation .P not only supports endometrial development but also potentially sustains the survival of the embryo . Luteal phase dysfunction (LPD) is associated with inadequate P production and consequent implantation failure. P supplementation is the most commonly used treatment when LPD can reasonably be assumed. Controlled ovarian hyper stimulation (COH) combined with intrauterine insemination IUI or invitro fertilization (IVF) are commonly used treatment protocols for couples with unexplained infertility UEI .. LPD and lack of P may also occur as part of assisted reproductive techniques ,including aspiration of granulosa cells or the use of gonadotropin releasing hormone (GnRh) analogs ; therefore luteal phase support(LPS) with P is a common practice in IVF cycles.The existence of LPD in stimulated IUI cycles is controversial subject.In COH cycles, multifollicular development and supraphysiologic steroid serum concentration may adversely affect LH secretion via a long-loop mechanism. Disturbed LH secretion may induce LPD with premature luteolysis, reduced luteal phase LH concentration, low P level and

shortened luteal phase . The previous studies have produced conflicting results and amount of data from well-controlled clinical trials is limited and further studies are required to characterize the impact of treatment with P for LPS in stimulated IUI cycles. The main aim of this study was to evaluate the effect of vaginal P (Cyclogest, 400mg progesterone, vaginal pessaries, Cox Pharmaceuticals, Barn staple, UK) on pregnancy and live birth rates in stimulated IUI cycles in UEI.

**Materials and Methods:** Single-center, prospective, randomized and blinded controlled trial in a tertiary care University fertility center, In 200 couples with UEI undergoing 511 consecutive stimulated IUI cycles, Clomiphene citrate and human Menopausal Gonadotropin (h M G) were used for ovulation induction. After IUI, the patients were randomized into the study group (n=98) received intra vaginal P (Cyclogest) for LPS. The patients randomized into the control group (n=102) received no LPS. Our outcome measures were Clinical Pregnancy rate (PR) and live birth rate (BR) per cycle and patient. Data were analyzed by Student, t and Chi-square tests.

**Results:** were no differences in demographic characteristics between the groups. Clinical Pregnancy rate per patient and cycle were similar in the study group (30.6% and 11.5%, respectively), and in the control group (25.5% and 10.03%, respectively) . There were no statistically significant increase in live birth rate per patient and cycle between the study group (19.4 % and 7.5 %, respectively) and the control group (14.7 and 5.7% , respectively).

**Conclusion:** Administration of vaginal P (Cyclogest) for LPS does not improve the reproductive outcome of stimulated IUI cycles.

**Keywords:** Clomiphene Citrate, human Menopausal Gonadotropin, Intrauterine Insemination, Luteal Phase Support, Progesterone, Unexplained Infertility

#### **P-55: Study of Day Three FSH and LH Level on Number and Quality of Oocytes in Infertility Women Candidate for ART Cycle**

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**Background:** Gonadotropins are main regulator of women menstrual cycles during which ovulation occur. infertile women with high level of FSH are poor responder to ovulation during ART cycles and do not take good result. this Study is done to evaluate the effects of day three FSH and LH level on number and quality of fertilized oocytes in infertile women candidate for ART cycle. Based on this study someone be able to have better prediction of the response of patient and results of treatment.

**Materials and Methods:** This is an experimental meta analysis on 59 women come to infertility center for ART treatment. In the third day of menstrual cycles , FSH and LH levels were measured with radioimmunoassay technique and their effects on quality and quantity of oocytes and also pregnancy rate was evaluated. FSH and LH amounts categorized in four groups and data examined by spss (16) software.

**Results:** Average levels of FSH= (9.01±8.7), LH=(7.56 ±7.27) and oocytes number was (7.88 ±10.29). FSH level had meaningful relationship with pregnancy rate, oocytes number,metaphase 2 oocytes number A quality oocytes and fertilized oocytes. LH level had no meaningful effect on the results.

**Conclusion:** In this study as FSH level increases the number and quality of oocytes , fertilized oocytes and pregnancy rate increase.the best result can be seen in FSH =10-15 miu/ml. LH increase also improve the effects and best results can be seen in 8≤ LH. In the other word maximum number of fertilized oocytes with grade A and grade B quality and least number of grade C quality is observed in this study. so it can be said that day three FSH and LH level can predict the results of ART cycles.

**Keywords:** Gonadotropin Hormones, FSH, LH, ART, Infertility

### **P-56: Effect of Metformin and Orlistat on Ovulatory Status in Obese PCOS Patients**

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**Background:** Polycystic Ovary Syndrome (PCOS) is the commonest cause of anovulatory infertility. Treatment modes available are numerous. The use of anti-obesity medications such as orlistat or insulin sensitizing agents such as metformin is sometimes indicated in these patients. We aimed to compare the effect of metformin vs orlistat on the hormone, lipid profile & ovulation status in obese PCOS patients.

**Materials and Methods:** A total of 80 obese women with PCOS were recruited in this prospective randomized study to receive metformin (n=40) or orlistat (n=40). Weight, BMI, waist, serum LH, total serum testosterone & lipid profile were assessed at baseline & after 3 months. Ovulatory status was assessed after 3 months.

**Results:** There was no significant difference between two groups regarding the ovulation rates ( p>0.05) . Treatment with both metformin & orlistat showed a significant drop in weight, waist, BMI (p<0.05), but there was no difference between two arms. Patients who was treated with orlistat, showed a significant drop in total testosterone, serum lipid. Patients who were treated with

metformin showed a significant drop in serum LH.

**Conclusion:** Both metformin & orlistat produce a similar effect on weight loss & ovulation rates.

**Keywords:** PCOS, Ovulation, Metformin, Orlistat

### **P-57: The Beneficial Adipokines in Reproduction and Infertility**

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**Background:** Adipokines are cytokines predominantly or exclusively expressed by adipose tissue that circulate and affect target tissues. Four known adipokines, adiponectin, visfatin/PBEF, omentin and vaspin, all increase tissue sensitivity to insulin, and are thus described as 'beneficial'. In this review such biological actions and potential roles of the adipokines leptin, adiponectin and resistin are explored in relation to female fertility and the complexity of the obese metabolic state.

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

**Results:** Adipokines are cytokines predominantly or exclusively expressed by adipose tissue that circulate and affect target tissues. Four known adipokines, adiponectin, visfatin/PBEF, omentin and vaspin, all increase tissue sensitivity to insulin, and are thus described as 'beneficial'. In this review such biological actions and potential roles of the adipokines leptin, adiponectin and resistin are explored in relation to female fertility and the complexity of the obese metabolic state.

**Conclusion:** Adipokines are cytokines predominantly or exclusively expressed by adipose tissue that circulate and affect target tissues. Four known adipokines, adiponectin, visfatin/PBEF, omentin and vaspin, all increase tissue sensitivity to insulin, and are thus described as 'beneficial'. In this review such biological actions and potential roles of the adipokines leptin, adiponectin and resistin are explored in relation to female fertility and the complexity of the obese metabolic state.

### **P-58: Secreted Frizzled Related Protein Type-4 as an Inducer of Apoptosis and Terminal Differentiation of Rat Granulosa Cells**

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**Background:** Involvement of Wnt proteins and one of its antagonist known as secreted Frizzled Related Protein type-4 (sFRRP-4) was reported in rodent ovarian follicular development. Other studies showed an ap-

optotic-associated expression of sFRP-4 and also additional non apoptotic-related function such as relationship with luteinization events in rat corpus luteum. This study sought to determine whether sFRP4 could be a direct inducer of apoptosis or terminal differentiation in rat granulosa cells by using recombinant human sFRP4 (rhsFRP4). To this order, the effect of rhsFRP-4 on sub-cellular localization of beta-catenin as an important mediator in Wnt/beta-catenin signaling and steroidogenesis was further examined.

**Materials and Methods:** Immature female rats were stimulated with PMSG (10 IU), ovaries were removed after 48h and granulosa cells were isolated mechanically. Cells were cultured in the presence of Testosterone (0.1 nM) and recombinant human FSH (50 ng/ml) for 48h named as FSH primed cells. Subsequently, FSH primed granulosa cells were treated with ovine LH (500 ng/ml) or rhsFRP-4 (0.5 ng/ml or 50 ng/ml) alone or both in combination for further 48h. Conditioned media were harvested after 48h or 96h for estradiol (E2) and progesterone (P4) detection by an ultrasensitive immunoassay enzyme linked assay. Subcellular localization of stabilized known as activated beta-catenin and its co-localization with active caspase-3 was further examined by using mouse monoclonal anti-activated beta-catenin antibody and rabbit polyclonal anti-active caspase-3 antibody which were assessed by double immunofluorescence method.

**Results:** Treatment of cells with rhsFRP4 alone or prior to FSH addition caused a significant increase of P4 secretion. However, using rhsFRP4 in combination with FSH or LH showed less potent effect on E2 or P4 secretion. Moreover, cell treatment prior to LH addition completely inhibit LH-induced P4 secretion. Interestingly, low dose of rhSFRP-4 strongly induced  $\beta$  catenin stabilization and its nuclear localization which was co-localized with nuclear active caspase-3 as revealed by double immunofluorescence.

**Conclusion:** Our data suggests that low concentration of sFRP-4 could play an agonistic role in Wnt signaling modulation by increasing beta-catenin stabilization and its subsequent nuclear localization which could be associated with apoptosis. Moreover, sFRP-4 could modulates differently granulosa cells FSH- and LH-induced steroidogenesis which may explain the involvement of Wnt signaling pathway in hormonal imbalance and infertility such as polycystic ovary.

**Keywords:** Granulosa Cells, Wnt/ $\beta$  Catenin Signaling, sFRP4, Apoptosis, Differentiation

### **P-59: Chlamydia Trachomatis Infection in Females with Secondary Infertility**

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**Background:** We designed a prospective study to assess the role of Chlamydia in secondary infertility

**Materials and Methods:** 40 women with secondary infertility and 30 term healthy pregnant women of similar age composition were studied for past and present chlamydia trachomatis infection. Women attending the outpatient department of Obstetrics and Gynaecology with complaint of secondary infertility were enrolled patients in the study. Chlamydia IgG was detected by ELISA, titres of 1:320 or more were considered positive. Endocervical swabs were collected for culture on cycloheximide treated McCoy cell lines and ELISA was used to detect chlamydia antigen. Hysterosalpingography was performed to assess tubal patency.

**Results:** IgG antibodies were present in 22(55%) females with secondary infertility while 2(5.5%) positivity was seen among controls,  $p < 0.001$ . Tubal occlusion occurred in 16(63.6%) cases positive for chlamydial antibody. Sensitivity of chlamydial IgG antibody as a diagnostic marker for infertility was 72.7% and specificity was 44.4%. Majority of chlamydia IgG antibody positive cases 17(77.2%) were symptomatic; Bad obstetric history was found in 16(72.7%) cases. Active infection was found in 12(30%) cases with one (3.3%) case of current infection occurring in the controls  $p < 0.01$ .

**Conclusion:** Prevalence of past chlamydial infection is strongly significant in women with secondary infertility. Current infection was also found significantly in these women. IgG antibody detection is an effective and non-invasive tool for detection of chlamydia and a more viable option in a developing country like India. Screening of women with secondary infertility for *C. trachomatis* is strongly recommended to allow early therapeutic interventions.

**Keywords:** Chlamydia Trachomatis, Secondary Infertility, IgG Antibodies, Chlamydia Antigen, Culture

### **P-60: Utilization of Acupuncture in the Treatment of Female and Male Infertility**

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**Background:** The use of complementary/alternative medicine (CAM) for health care has been increasing including the use of acupuncture for the treatment of infertility. The aim of this study was to review existing scientific rationale and clinical data in the utilization of acupuncture in the treatment of female and male infertility.

**Materials and Methods:** Medline, Science Direct computer search was performed to identify relevant articles about the utilization of acupuncture in the treatment of female and male infertility.

**Results:** The peripheral impact of acupuncture in improving uterine artery blood flow and hence endometrial thickness also provides encouraging data regarding its potential positive effect on implantation. Paulus et al. reported that the women who received 25 minutes of acupuncture before and a gain 25 minutes after embryo transfer had a 42.5% clinical pregnancy rates (PR) compared to a 26.3% rate ( $p=.03$ ) in the control subjects who laid quietly for an equivalent amount of time. Domar et al. demonstrated that the use of acupuncture in patients undergoing IVF was not associated with an increase in PR however; they were more relaxed and more optimistic. Numerous researches of acupuncture treatment on infertile men have also been conducted. Reports from uncontrolled trials using acupuncture on infertile men have indicated that a positive effect on sperm concentration and motility, an increase in testosterone, and some improvement in luteinizing hormone (LH) level. These studies have also shown enhance of normally shaped sperm and a significant decrease in the percentage of morphologically abnormal sperm. Some studies also have implied that acupuncture did not trigger subjective behavior alterations or influence sexual behavior.

**Conclusion:** The treatment of idiopathic male and female infertility could benefit from employing acupuncture. Prospective randomized controlled studies are needed to evaluate the efficacy of acupuncture in female and male fertility treatment.

**Keywords:** Acupuncture, Treatment, Infertility

### **P-61: Increase Stress Level in Infertile Women that Referred to Infertility Clinic of Zeinabieh Hospital of Shiraz**

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**Background:** Infertility is defined as the inability of a couple to become pregnant after one year of unprotected intercourse. Now that health care professionals developed the ability to diagnose and treat most cases of infertility, infertility viewed as a organic condition. However, there is evidence that stress levels influence the outcome of infertility treatment and treatment failure, but the rule of stress and anxiety in infertility is not yet clear. Our aim in this study is to explore the psychological consequences of infertility on couples' life.

**Materials and Methods:** Our study conducted at March and April 2010 at infertility clinic of Zeinabieh Hospital. We prepared questionnaire with six main questions, all of questionnaire completed by 125 women that were treated for infertility in infertility clinic of Zeinabieh Hospital.

**Results:** Psychological stress, depression and anxiety appears to be more common in the partner with the fertil-

ity problem and level of stress in infertility patients tends to increase during treatment period. Also sexual disorders after diagnosis and medical therapy are common in infertile women. Most of them (85%) had no counseling during treatment.

**Conclusion:** We suggest interventions (for example: relaxation techniques, stress-management, coping skills training, and group support) to relieve stress in infertile couples these can increase rate of pregnancy; Also couples can be more comfortable during treatment. Evaluation of patients with more symptoms by psychiatrist can be effective.

**Keywords:** Infertility, Stress, Treatment

### **P-62: Infertility and PCOS**

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**Background:** Polycystic ovary syndrome (PCOS) is one of the most common endocrine metabolic disorders of reproductive age women. The main signs of PCOS are as follows: androgen excess, menstrual dysfunction, infertility, obesity, and other numerous health problems. By different authors, the disorder affects 2-28% of reproductive age women.

**Materials and Methods:** Polycystic ovary syndrome is characterized by presence of hyperandrogenism, anovulation, menstrual cycle disturbances, also by the other metabolic changes. The lack of well-defined and universally accepted diagnostic criteria makes identification of this syndrome confusing to many clinicians. There are only few studies concerning the correlations between phenotypic expression, body composition and PCOS, and relationship with the processes of growth and sexual maturation and various environmental factors (nutrition, physical activity, stress, and other factors).

**Results:** There is a lack of knowledge about further PCOS development and prognosis, considering the individual and environmental factors. Variation in human body composition and shape ranges considerably: many body size and shape indices (height, weight, body composition, and proportions) are the result of long evolution process and adaptation to environment. Obviously, the morphological body parameters, physiological and biochemical indices are complex and compound the interdependent system. By current literature, more than 50% of women are overweight or obese. If waist circumference and waist-to-hip ratio of women with PCOS increase, reproductive function and metabolic state of a woman is altered more than in cases when there are no changes in these parameters. The investigations of the strongest sexual dimorphism sign--the subcutaneous and visceral fat topography--showed that women with PCOS have greater adipose tissue mass in the areas of the abdomen, waist, and upper arms than control women.

**Conclusion:** It is known that some indices of sexual dimorphism may be considered as the morphological signs of hyperandrogenism, for example handgrip, waist-to-hip ratio, hand and foot length, 2nd-to-4th digit (finger length) ratio (2D:4D), certain facial characteristics. Only 2D:4D ratio was investigated for the women with PCOS. The early changes of certain morphological and other indices of physical status could help to predict some metabolic characteristics, development of PCOS, and outcome of this syndrome.

**Keywords:** Infertility, PCOS

### **P-63: The Comparison of Psychological Aspects of Infertile Males and Females**

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Infertility is a relatively prevail problem which has been considered as a source of psychological distresses in many studies. The period of infertility, the type of treatment offered, the person's ability in coping, prognosis of infertility factors, psycho – emotional supports and social supports available are among different factors that influence the psycho – social function of the individuals. The results of the studies done by psychologists, clinical and social psychologist as well as mental health professionals have shown that the infertile persons who are under the chronic stress are more prone to get disease like depression, anxiety, low self steams and dissatisfaction. In general, the experience of stressful events cause low control in person's life and forces him or her to get into anxiety disorders. It also cause aggressive behavior, the feeling of helplessness, disappointment, embarrassment, anxiety and problem in sex needs is one of the factors that influence the process and reaction of the person toward infertility. In studies done by Connolly and et al. (1992), Burnstein and et al. (1988) the results have shown that males and females are different. This experience is more stressful in females than in males. The infertile females, in comparison with infertile males experience more psychological problems. Also infertility has a more negative meaning for females than for males, Bery and Wilson (1991), Shover and et al. (1992), Greil(1991). In this sort of studies, it has been concluded that females, in comparison with infertile males: 1- Start treatment sooner and stop it later. 2- Look for the information related to infertility in the environment around more. 3- Having babies is considered as the only treatment of infertility negative outcomes. 4- Have low self steam. 5- Have more depression. 6-

Have low life satisfaction. 7- Consider infertility as an unacceptable thing. 8- They blame themselves for the infertility problem. What we should know is that the difference in sex and its effect on infertility is more important than the problem person. The role of sex (males or females) is of great importance in infertility. What we should consider more is the psychosocial aspects of infertility.

**Keywords:** Psychological Aspect of Infertility, Sex Differences, Infertility

### **P-64: Strategies to Optimize Reproductive Efficiency by Regulation of Ovarian Function in yak (Poepagus Grunniens L.)**

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**Background:** To design a pre-Ovsynch hormonal strategy in yaks in order to increase the ovulatory response to the first GnRH injection of Ovsynch so that overall synchronization rate to Ovsynch could be improved.

**Materials and Methods:** Non-lactating cyclic yak cows (n=33) were assigned to receive either no treatment before Ovsynch (control) or 0.375mg of PGF2(PreP) followed 2 d later by 10 g of GnRH (PreG), administered 4 (G4G), 5 (G5G), or 6 (G6G) d before initiating the Ovsynch protocol. Rectal palpation was performed to assess ovulation and blood samples were collected to measure progesterone concentrations during pre-treatment, treatment and post-treatment periods. All the animals received timed AI 12 and 24 h after the final GnRH of Ovsynch. Diagnoses for pregnancy were performed by rectal palpation and profiles of plasma progesterone 35 d after AI.

**Results:** Percentage of yak cows that ovulated in response to the first GnRH injection of Ovsynch, synchronized to Ovsynch treatment, had a functional CL at PGF2 of Ovsynch, had circulating concentrations of P4 at PGF2 of Ovsynch and likely to be pregnant after 35 d after AI, were greater in G6G and G5G compared with control, whereas G4G did not differ from controls. In addition, animals that ovulated in response to first GnRH of Ovsynch had greater response to PGF2 of Ovsynch and greater synchronization rate to the overall protocol than those that did not ovulate.

**Conclusion:** To conclude, PGF2,-and-GnRH-based pre-Ovsynch strategies consisting of 5 or 6-d interval between PreG and first GnRH of Ovsynch resulted in a greater ovulatory and luteolytic response to first GnRH and PGF2 of Ovsynch, respectively, compared with control animals. These, in turn, optimized synchronization rate to Ovsynch in yaks.

**Keywords:** Yak, Ovsynch, Ovulation, Estrus, Synchronization

**P-65: Efficacy of Chamomile- Extract on Polycystic Ovary Syndrome in Rat**

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**Background:** Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder associated with ovulatory dysfunction. Presently, little is known about the primary factors that initiate the PCOS dysfunction. Chamomile flowers are used in alternative medicine for its anti-spasmodic and inflammatory effects. Antispasmodic properties of chamomile ease menstrual cramps and lessen the possibility of premature labor. This medicinal herb also stimulates menstruation. In this study, we evaluated the effects of Chamomile alcoholic-extract on the biochemical and clinical parameters in a rat model of PCOS.

**Materials and Methods:** Estrous cyclicity of 30 virgin adult cycling rats was monitored by vaginal smears obtained between 0800 and 1200 hours. After about 4 days each received an i.m. injection of Estradiol Valerate (Aburaihan Co., Iran), 2mg in 0.2 ml of corn oil, to induce PCO. Control rats were injected with corn oil. All the rats in the experimental group were evaluated for follicular cysts 60 days after the injection. Later, the rats with PCOS were treated by multiple doses (25, 50, 75 mg/kg) of intraperitoneal injections of Chamomile alcoholic-extract for ten days. SPSS 13 was used for statistical data analysis and data, were analyzed by ANOVA, followed by the Student- Newman- Keuls Post hoc test  $p < 0.05$ .

**Results:** The histological and hormonal results showed that Chamomile can decrease the signs of PCOS in the ovarian tissue and help LH secretion in rats ( $p < 0.05$ ).

**Conclusion:** The alcoholic-extract of dried *Matricaria chamomilla* L. flowers can not only induce recovery from a PCO induced state in rats, but also increase dominant follicles. Additionally better endometrial tissue arrangements were also observed in the uterine tissue; as another therapeutic effect for Chamomile.

**Keywords:** Polycystic Ovary Syndrome (PCOS), Chamomile, Extract, Estradiol Valerate (EV), Rat

**P-66: Opportunities of Assisted Reproductive Treatment in Turkmenistan Initial Results**

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**Background:** It has not only medical, social and demographic but also economic aspects. The policy of the President of Turkmenistan Gurbanguly Berdimuhamedov on public health services development is directed on granting to patients of high quality medical services. In this connection in Turkmenistan, as well as all over the world, since 2007 launched the use of IVF technology in the joint Turkmen-German project with Dragon IVF International Company.

**Materials and Methods:** Simple *in vitro* fertilization; Sperm insemination of husband or donor; Induction of super-ovulation; Embryo transfer into uterine cavity.

**Results:** From March 2007 to March 2010 performed only 153 cases of assisted reproductive treatment. Including usual IVF - 61 cases, fertility indicator - 58.4%, tested pregnancy - 39.1%; ICSI - 105 cases, fertility indicator - 72.0%, tested pregnancy - 48.6%.

**Conclusion:** The obtained data are comparable to literature. Thus, advanced reproductive treatments increase chances of patients at infertility treatment.

**Keywords:** Infertility, Male and Female Factors, *In vitro* Fertilization, Sperm Insemination, Induction of Super-ovulation

**P-67: The Comparison of the IVF Outcome between Three Methods of Induction Ovulation in PCOS Patients**

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**Background:** Polycystic ovarian syndrome (PCOS) is the most important cause of oligoovulation and anovulation in women of reproductive age and in infertile women. Several approaches have been proposed to induce ovulation in pcos patients and gonadotropins are the classical and effective therapeutic options. This study was carried out with the Backgrounds of comparing of fertility rate with anly FSH, protocol, HMG protocol and combination of FSH and HMG for induction ovulation.

**Materials and Methods:** 95 infertile women, by pcos diagnosis, in accordance with Rotterdam criteria who had been admitted to Montaserieh infertility center in mashhad, were selected 25 patients were induction ovulation by recombinant FSH, 35 patients by HMG, and 35 patients by a combination of FSH and HMG. Assessment was done in the ninth day of the menstrual cycle and the day of HCG injection by intravaginal sonography. The mean size and number of follicles in each ovary and the endometrial thickness was measured and recorded. After retrieval of the oocytes and incubating with sperms in lab was done the embryo transfer, two weeks after the fertilization the  $\beta$ HCG test was done and if the test

was positive, transvaginal ultrasound was done for FHR after 6 weeks. And if the result was positive fertility would be confirmed. After data collection and entry statistical analysis (t test, ANOVA and chi square test) on the basis of the hypothesis, with a significant level of  $p=0.05$  was performed using SPSS software.

**Results:** Considering the response to treatment endometrial thickness, mean number and size of follicles in the ninth day of menstrual cycle and day of HCG injection are the same. Only the mean numbers of follicles in the right ovary in the ninth day of menstrual cycle in three groups were significantly different ( $p=0.032$ ). Other variables in three groups did not show a significant difference.

**Conclusion:** In this study, the result of treatment of PCOS patients using induction ovulation methods such as FSH, HMG, or combination of them, demonstrated no differences in fertility outcome. Although fertility rate of FSH group was more than of other groups, this might be attributed to the increased level of endogenous LH in PCOS patients.

**Keywords:** Polycystic Ovary Syndrome, Rec FSH, Human Menopausal Gonadotropine, Fertility, Pregnancy, Induction Ovulation

### **P-68: Women's Infertility in Ilam Province; Characteristics and Associated Factors**

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**Background:** Infertility or not having a baby a year after marriage without using contraceptive methods is the most common complication in both developed and devaluing countries. Approximately 25-15% of couples are experienced infertility in their life. The present study aimed to determine the characteristics and associated factors with women's infertility in Ilam province western Iran.

**Materials and Methods:** In this descriptive-analytical study, 48 women with infertility problem who referred to the gynecologic clinic were recruited. Demographic and clinical data were collected using a valid and reliable questionnaire containing two sections on health history of subjects themselves and their partners were completed by the interview. SPSS was used for all analysis.

**Results:** The mean age was  $27.9 \pm 6.2$  years and 35/4% were living in rural areas. Mean marriage age and age at menarche were  $21.5 \pm 4.8$  and  $14.1 \pm 2.4$  years respec-

tively. Overall, 34% of subjects were less educated. The mean age of partners and marriage age, were  $32.6 \pm 6.8$  years and  $26.2 \pm 4.4$  years respectively. Two third (75%) had primary infertility and the rest of one third had secondary infertility. 58.7% of subjects and 50% of partners were either overweight or obese. Overall, 31.2% had complained of irregular menstruation, 33.3% had a history of consanguineous marriage and 12.5% reported an infertility history in their closed relatives (mother and sister). Majority of the subjects (68.2%) had used contraceptive methods including LD pills in particular. History of abortion was reported by 10.4%. Pelvic infections (45.8%), hirsutism (12.5%), weight changes (12.5%), thyroid disorders (10.4%) and decreased libido (8.3%) were documented as associated factors with infertility. The subjects' partners were also reported history of testicular surgery (12.5%), inflammatory bowel disease (6.2%), decreased libido (6.2%) and history of contact with X-ray or chemotherapy (4.2%). A significant relationship was observed between mean marriage age and irregular period menstruation as well as decreased sexual desire ( $p<0.002$  for both). There was also a relationship between the irregular period menstruation and pelvic infection ( $p<0.04$ ).

**Conclusion:** It seems that cultural factors, consanguinity, diets, sedentary lifestyle leading to overweight and obesity were the major factors associated with infertility in this region. The role of clinical factors such as pelvic infections and irregular period menstruation also cannot be ignored. Further case-control studies and clinical trials are recommended to determine the exact factors affected women's infertility.

**Keywords:** Infertility, Women, Ilam

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## **Genetics**

### **P-69: Spermatogenic Activity of Aloe Vera; Increasing Sperm Parameters and CREM Gene Expression**

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**Background:** Infertility affects 15% of couples around the world. Male factor accounts for 40-50% of conjugal infertility. cAMP responsive element modulator (CREM) is a key factor in the regulation of the expression of number of postmeiotic genes that control spermatogenesis. Aloe vera is a medicinal plant used to treat several diseases. The purpose of this study was to investigate effects of Aloe vera extract on rat spermatogenesis with emphasis on sperm parameters, hormonal assay, and CREM gene expression in testis tissue.

**Materials and Methods:** Aloe vera leaf pulp extract,

gel extract, and a mixture of both administered to three groups of 10-week old male Wistar rats for 56 consecutive days. Then sperm analysis, hormonal assay, and RT-PCR were carried out.

**Results:** Results indicated that in all groups except control group the weights of the testes have increased. Epididymal sperm counts and sperm motility have been increased significantly compared to control groups. Analysis of testosterone level between groups showed that the level of this hormone in the groups that treated with Aloe Vera has increased remarkably. Results of RT-PCR showed an increase in the CREM gene expression in the groups that received gel extract and mixture of gel and leaf pulp extract.

**Conclusion:** According to the results of this study, Aloe Vera has strong spermatogenic activity by increasing sperm parameters and CREM gene expression. This study strongly proposes that Aloe vera specially its gel fortifies spermatogenesis and can be a good candidate for manufacturing fertility drugs.

**Keywords:** Spermatogenesis, CREM Gene Expression, Aloe Vera

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## Reproductive Imaging

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### P-70: Ultrasound Guided Artificial Insemination: A Randomized Controlled Trial

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**Background:** To ascertain whether using ultrasound guidance during intrauterine insemination (IUI) could increase pregnancy rates.

**Materials and Methods:** The population under study consisted of 73 consecutive couples subjected to IUI in our Human Reproduction Unit, between September and December 2006, with a total of 231 IUI cycles performed. The patients were randomized using a computer-generated, random numeric table, in two groups: ultrasound-guided IUI group (n=33) and clinical IUI group (n = 40).

**Results:** The pregnancy rate was 16.04% per cycle in ultrasound guided IUI and 16.80% in control group; no statistically significant differences were observed between the groups. There were no differences in pregnancy rate per woman, nor in first cycle pregnancy rate. The cumulative pregnancy rate was also similar in both populations. There were no differences in pregnancy rate according to the different cervico-uterine angles.

**Conclusion:** Ultrasound-guided intrauterine insemination does not produce better results than blind insemination, since the pregnancy rate per cycle is similar.

**Keywords:** Intrauterine Insemination, Pregnancy Rate, Ultrasound

### P-71: Valuation of Vascularization in Cows with Follicular Cysts after Epidural Administration of a GnRH Analogue

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**Background:** The aim of this study was to investigate the effects of administration of a GnRH analogue to cows with ovarian follicular cysts on plasma renin concentrations and ovarian vascularization.

**Materials and Methods:** This study was performed with 60 Friesian cows, which were diagnosed with follicular cysts, and randomly allocated into two groups: group A (treatment; n = 30) received 2 ml of lecorelin (Dalmarelin® – Fatro), per head via sacro-coccygeal epidural, and group B (control; n = 30) received 2 ml saline solution (0.9% NaCl) per head by the same route. Blood samples were immediately collected prior to administration (T0) and then 24 h (T1), 48 h (T2) and 8 days (T3) after administration of the treatment, for both groups. Ovarian vascularization was evaluated utilizing Power Doppler on these same days in 10 animals from each group. The number of pixels detected by Power Doppler was used as an indicator of the degree of vascularization.

**Results:** Plasma renin concentrations remained relatively constant for the control (group B) animals, but increased as the sampling period progressed (NS) for the treated cows (group A). Similarly, there were no changes in ovarian vascularization (number of pixels) for the control cows, but vascularization increased throughout the sampling period in the treated animals. The number of pixels associated with cysts was significantly higher for treated compared to control cows at 24 h after treatment (p<0.001).

**Conclusion:** The epidural administration of a GnRH analogue was determined to be a highly effective therapy for follicular cysts (regression occurred in 82% of treated cows within 8 ± 2 days after treatment, but in none of the control cows), which also enhanced ovarian vascularization

**Keywords:** Renin, Cow, Ovarian Vascularization, Power Doppler, Follicular Cysts

Abstracts of

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## Oral Presentations

### O<sub>nm</sub>-1: Imaging in Female Infertility

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The role of imaging in female infertility has undergone a revolution over the past few decades. Sonography plays an integral role in the evaluation of gynecologic disease. It can determine the organ or site of abnormality and provide a diagnosis or short differential in the vast majority of patients. Both the transabdominal and transvaginal approaches are now well-established techniques for assessing the female pelvic organs. Transvaginal sonography is now considered an essential part of almost all pelvic ultrasound examinations. Color and spectral Doppler sonography have evolved to play a role in assessing normal and pathologic blood flow. Doppler can also distinguish vascular structures from nonvascular structures, such as dilated fallopian tubes or fluid-filled bowel loops. The more recent addition of Hysterosonography (SHG) has provided more detailed evaluation of the endometrium, allowing differentiation among intracavitary, endometrial, and submucosal lesions. Three-dimensional (3D) ultrasound also plays an important role in the diagnosis of congenital uterine anomalies and endometrial volume measurements.

Sonography also plays an important role in guiding interventional procedures such as magnetic resonance imaging (MRI). MRI because of excellent tissue characterization, can occasionally be helpful when sonography is inconclusive and in the staging of pelvic malignancies. Computed tomography (CT) has a limited role but is used for cancer staging. Hysterosalpingography (HSG) is the radiographic evaluation of the cavity of the uterus and fallopian tube and as a commonly performed examination plays an important role in reproductive medicine.

### O<sub>nm</sub>-2: Obesity and Female Fertility

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**Background:** Infertility & Obesity have often been linked among of reproductive age. Obesity results in an increased production of Estrogen and Androgen, these hormonal imbalances interfere with ovulation, which of course is the basis of successful conception.

Obesity is a characteristic of polycystic ovary syndrome (PCOS). Obesity is linked to several health conditions, including cardiovascular disease, Hypertension, Diabetes.

In obesity assessment it is necessary to pay attention to

other disease such as thyroid dysfunctions, fatty liver, and hyperlipidemia before pregnancy. As well as request some blood test that is needed and refer to the related specialist if necessary.

Women affected by obesity not only have problems with fertility but are also at a greater risk for pregnancy complications such as having Caesarean Section, giving birth to a large baby, Gestational Diabetes. There are many options available in treating obesity such as: Altering diet, Exercises regularly, Medication & different kind of surgical methods.

**Materials and Methods:** We do our study on 172 subjects committed Nutrition Unit in Royan Institute during 6 months that divided in two groups 88 were PCOS & 84 NonPCOS.

We measures BMI, Waist and Hip circumference. BMI is calculated by dividing a person's weight (in Kg) by his or her height (in meters squared). Waist and Hip circumference by measuring the smallest circumference of the natural Waist, usually just above the belly button, and dividing by the hip circumferences at its widest part of the buttocks or hip. Also we requested for all patients (TSH, SGOT, SGPT, LDL, HDL, TG, and Cholestrol).

**Results:** Totally all subjects had approximately 8 Kg weight loss indurations 6 month. (MAX: 3 Kg, MIN: 24 Kg).

No significant difference in comparisons between the average of the Lab variants, Waist and BMI were seen between PCOS and nonPCOS group, but TSH ( $p=0.02$ ) and history of hyperthyroidism (16.7% versus 2.3%) were so higher in nonPCOS group, on the whole 19% of Nutrition Clinic's patients had a history of hypothyroidism that indicate our attention to thyroid dysfunction in obese patients. Also liver enzymes dysfunctions in PCOS group was 11.4% and in nonPCOS was 4.2% that in PCO group possibly it was because of fatty liver resulting from insulin resistance. Additionally Chol-LDL > 130 mg/dl range in PCOS group was so higher (25% versus 14.6%).

**Conclusion:** Studies have shown that modest weight loss can have a significant effect on women with PCOS, often resulting in a regular menstrual cycle and fertility. Ideally weight loss should be accomplished prior to a conception & pregnancy. It is believed that weight loss prior to pregnancy may significantly decrease maternal & fetal risks associated with pregnancy. Additionally study exhibits the important measurement of TSH, Liver function and Lipid profile tests in obese patients who referred to nutrition clinics.

### O<sub>nm</sub>-3: Embryo Transfer Performed by a Midwife in a Prospective Randomized Study

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More than 15 % of couples have infertility problems. The

causes of infertility are equally distributed among men and women. Assisted reproduction is an option which can help many of these couples. Because of ongoing research and development of the infertility treatments the pregnancy outcome after in vitro fertilization can be increased.

Embryo transfer (ET) has in the Nordic countries, by tradition been performed by a gynecologist. As the gynecologists often have a busy schedule, midwives and nurses have become increasingly important in planning the treatment, providing subjects information, ultrasound monitoring and assistance at ET. As part of the continuous development of our IVF programmer, we have carried out a prospective randomized study where either a midwife or a gynecologist has performed ET. The objective of this study was to investigate if an experienced IVF midwife could perform ET with similar results as a gynecologist. The result show that the clinical pregnancy rates between ETs performed by midwives versus gynecologists was 31 % and 29% respectively. The patients were satisfied when a midwife performed ET as reported in questionnaire. Our conclusion was that it is a feasible option to allow midwives to carry out ETs.

#### **O<sub>nm</sub>-4: Reproductive Factors Related to Cancer of the Breast, before and after Menopause; among Women Referring to Health Centers in Tehran, 2005**

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**Background:** To find out those reproductive factors to incidence of breast cancer overall and by menopausal status.

**Materials and Methods:** This was a case – control study, in which, the risk of menstrual, pregnancy- delivery and menopausal factors were determined in women with breast cancer (case) against those without (control). The risk factors stratified by menopausal status also. A total of 500 women, diagnosed with breast cancer, (250 in premenopausal and 250 in post menopausal status), were selected from shohada hospital and cancer institute in Tehran, and 500 women as controls visiting patients at the same time or living next door to cases.

**Results:** The results showed that early menarche (OR=17.26, p=0.000), breast feeding less than 3 months in life (OR=2.73, p=0.000) are overall risk factors for breast cancer and both at pre (OR=15.61, p=0.000 and OR=1.77, p=0.000), and postmenopausal (OR=0.05, p=0.003 and OR=0.28, p=0.001). Menstrual interval less than 26 days, and irregular menstruation period was related to breast cancer (OR=0.30, p=0.000 and OR=2.39, p=0.026 respectively) and in premenopausal period (OR=0.25, p=0/000 and OR=2.71, p=0.017 respectively). On the other hand, irregular menstrual period in the age of 30, time interval more than 20 years between

menarche and first pregnancy were related to breast cancer (OR=18.66, p=0/000 and OR=0.45, p=0.000 respectively). We also found that two mentioned variables were risk factors of breast cancer in post menopausal period (OR=0.02, p=0.000 and OR=2.85, p=0.000 respectively). This study showed that overall, cancer of the breast is statistically related to scanty menstrual bleeding (OR=1.72, p=0.000) and occurrence of first pregnancy after age of 20 (OR=2.40, p=0.000), history of post term delivery (OR=3.15, p=0.038), history of 2 spontaneous abortion (OR=2.16, P=0.043), history of induced abortion (OR=2.03, p=0.018), and the usage of oral contraceptive pill more than 5 years (OR=0.40, p=0.001) were only related to premenopausal breast cancer. It was also shown that, dysmenorrheal (OR=2.24, p=0.027), marital age after age of 30 (OR=3.79, p=0.011), hot flashes at menopause (OR=0.32, p=0.004) and occurrence of menopause after the age of 55 (OR=3.84, p=0.000) were only related to breast cancer of post menopausal status.

**Conclusion:** Regarding our results the age in which the disease is diagnosed is a prominent point for sow factors. It can be assumed that the most important factors are irregular menstruation at the age of 30 for both pre and post menopause. Meanwhile, early menarche for premenopausal breast cancer and menopausal age for post menopausal breast cancer were considered to be the most important factors.

**Keywords:** Breast Cancer, Reproductive Factors, Age of Diagnosis

#### **O<sub>nm</sub>-5: Patient Management**

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Infertility is defined as failure to conceive after 1 year of unprotected intercourse (6 months for women over 35). The prevalence of infertility appears to be increasing; many women are postponing childbearing for social, professional, financial, or psychological reasons. The current evidence indicates a 9% prevalence of infertility (of 12 months) with 56% of couples seeking medical care. 80% of all women desiring children conceive within 1 year of marriage and another 10% within the second year. In the late 20th century, medical science has made great advances in understanding each stage of the reproductive process and in identifying the problems that can occur at each step. In an increasing number of cases these barriers can be corrected or worked around in order to achieve fertility for about 65% of couples who seek the help of fertility specialists. Despite public worry

and discussion, the actual incidence of infertility has remained fairly stable over the years. One American couple out of 5 or 6 currently experiences infertility. Infertility grows more common with increasing age; about 33% of couples in their late 30s are infertile. The age factor has taken on new importance as many people in the United States and similar industrialized countries have put off marriage and children until certain educational or career goals are reached.

Increased awareness and availability of modern treatments that assist couples to conceive and the decreased supply of infants for adoption have led more couples to seek infertility therapy. While the inability to conceive distresses many couples, they differ in their willingness to undergo intensive investigation and treatment for infertility.

All women should be aware of certain information before trying to conceive. It is also important to counsel women about smoking cessation, weight control. Natural fertility declines with age. The main causes of infertility are related to ovulatory dysfunction; blocked or damaged fallopian tubes; and abnormalities of sperm number, motility, or morphology. Questions should focus on four main areas: ovulatory dysfunction, risk factors for tubal infertility, sexual factors, and male or sperm factors. Physical examination of women includes assessment of body mass index, thyroid, breasts, and signs of hyperandrogenism. A Pap smear should be done if indicated, along with a bimanual examination to search for signs of endometriosis or pelvic adhesions, such as a fixed retroverted uterus, adnexal masses or tenderness, and uterosacral ligament thickening, nodules, or tenderness. Infertility investigations aim to assess three main areas: ovulation, tubal damage or dysfunction, and male factors. First-line investigations generally include a semen analysis and assessment of tubal patency, usually by HSG. Semen analysis is readily available in most communities.

The need for blood tests is determined by the history; a battery of tests is rarely required. Routine random measurement of FSH, TSH, and prolactin. A woman with a suspicion of chronic anovulation most probably due to polycystic ovary (PCO) syndrome, as there is a long history of irregular cycles and clinical presentation with hirsutism, her serum levels of testosterone hormone, SHBG, DHEA, DHEAS and prolactin should be evaluated to prove the provisional diagnosis and to detect the source of excess androgens.

Semen analysis is best performed after 72 hours of abstinence. A longer period of abstinence results in increased sperm count, but reduced motility.

There are three main types of fertility treatment: medical treatment (such as ovulation induction therapy); surgical treatment (such as laparoscopy and hysteroscopy); and the different assisted reproduction techniques (ART) such as IUI, IVF, ICSI, IVM. Choice of infertility treatment often related to issues of efficacy, cost, ease of use or administration, and its side effects. Legal, cultural and religious inquiries have limited the available choices in some countries, such as the use of donor sperms or oocytes.

Treatment options available for any particular infertile couple will depend also on the duration of their infertility, which partner is affected, the age of the female partner. It is customary to transfer more than one embryo to the uterus to increase the chance of at least one embryo implanting; the risk of multiple pregnancies must be balanced with the chance of achieving a pregnancy at all. It is not appropriate to replace more than two embryos in women under the age of 37. High multiple pregnancies are much more likely to be the result of inappropriate ovulation induction in polycystic ovarian syndrome than they are of in vitro fertilization and embryo transfer.

Fertility clinics should address the psycho-social and emotional needs of infertile couples as well as their medical needs. The content of counseling may differ depending on the concerned couple and the existing treatment options. It usually involves treatment implication counseling, emotional support counseling, and therapeutic counseling.

Infertility by itself does not threaten the life, but it has devastating psycho-social consequences on infertile couples. It remains a worldwide problem challenge. Management of infertility has been and still a difficult medical task not only because of the difficulty in the diagnosis and treatment of the reproductive disorders in each partner, or the poorly unstated interaction between the partners' fertility potentials, but also because of the fact that success of treatment is clearly identifiable entity; the achievement of pregnancy. The treating doctor who is counseling the couple regarding their infertility must be familiar with the causes, investigations and the treatment options available. The couple needs to be given realistic information about their chances of having a live birth, as well as, the risks and costs of the management plan and its alternatives.

#### **O<sub>nm</sub>-6: Apoptosis and the Quality Decrease Of Sperm Parameters in Infertile Men.—Meta Analysis**

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**Background:** The main objectives of this study are to examine the Meta analysis of the relation between apoptosis and decline of sperm motility, concentration and semen volume in infertile men. Through testis biopsy, high amounts of apoptosis have been reported. Since the results of sperm analysis shows a medium prediction of fertility. Infertility due to male factor is 30%-40% of infertility of couples, so clinical evaluations and new methods in male factor can be a more accurate prediction for the condition and function of their sperm, therefore sperm apoptosis can be a useful index for evaluat-

ing male fertility.

**Materials and Methods:** An internet study, by the use of Medline and Scopus database from 2003 to 2009 was performed. The key words were sperm apoptosis and its quality. This study is done on human subjects. The selected articles were not biased or had the minimum bias. Among the 20 initial articles, only 9 had the wanted characteristics and were chosen. The results were obtained from a Random-Effects model which is more conservative than a fixed-Effect model. The homogeneity of the effects was performed by the use of Cochran's Chi-squared (Q-Test) and I<sup>2</sup> statistics. The homogeneity of the effects depends on the type of variables which evaluate the apoptosis and quality of sperms. The effects of small study and low quality of the study were done through cumulative Meta analysis and analysis of sub groups. Metaregretation was done for recognizing the Covariance of the study level and its Heterogenic. The bias of study was done through Funnel plot. CMA statistical software is used in this Meta analysis.

**Results:** This results show a significant difference between motility of sperm in two groups. ( $p=0.000$ ) also based on Random model the concentration of sperm in control and case groups are significant differences. ( $p=0.001$ ) but there is not a significant deference between semen volume and intensity of apoptosis ( $p=0.275$ ).

**Conclusion:** Apoptosis and its intensity can affect the motility and concentration of sperm therefore can be affect sperm parameters.

**Keyword:** Apoptosis, Sperm Quality, Infertility

### **O<sub>nm</sub>-7: Psychosocial Aspect of Infertility**

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**Background:** A common wish which is found in almost all humans is desire for having a child. Infertility depression is considered as life and identity crises. The intertwined network of physical, mental, and social factors affect the infertile person. The elements, relationship and activities of this complex network determine the way the person responds to the problem of infertility. Beside all the factors that lead to depression, the infertile people are more susceptible to depression due to specific factors related to their infertility. It is expected that demographic factors, as a part of social background, are effective in how an individual meets the problems resulted from infertility. Beside demographic issues marital satisfaction is another factor which is effective in life satisfaction and nervous health of infertile couples. This research is designed in order to study the relation of demographic factors on the nervous disorders in the fertile women as well as identifying the relation of sexual disorder and infertility.

**Materials and Methods:** This research is a qualitative-quantitative study. In this study, 100 infertile women were selected through simple random sampling. They were studied by means of two questionnaires, MHQ and

Sexual Disorder Questionnaire. The collected data were analyzed with SPSS software by t test and statistical analyses.

**Results:** The results of this research indicated that there is no significant relationship between the age of the infertile women and their anxiety and depression score (the average age of the subjects was 31.2 years old.) there was a significant relationship between the education level and attitude towards infertility. The subjects with very low level of education (primary school) and those with high level of education (master degree and higher) were affected more. In subjects with high level of education the type of attitude they had toward infertility caused them to lose their social reputation, instability in their social status, and the feeling of being belittled in the society. Occupational and high income of the espouse increased confidence of the women under costly treatments. Most of these couples were sensitive toward attitudes of their relatives and associates. The women who lived in the house of one of their parents had higher level of depression. Change of attitude in the infertile women after diagnosis had affected sexual relations in the following ways: dissatisfaction with sexual relations (50%), disorder in the couples' relations (47%), reduction of intercourse (32%), Libido (67%), lack of orgasm in more than half of sexual intercourses (60%). 57% of infertile women suffered from depression and 67.2% suffered from anxiety.

**Conclusion:** Infertility is not a merely medical problem but it faces many of infertile couples with crisis in different aspects of their lives. Identifying prevalent emotional problems among these couples, offering consultation services in the first visit considering the cultural, educational, and social level of couples, establishing sexual consultation centers in the clinics of infertility, nutritional consultation, and improving the quality of life along with medical treatments are considered important in order to reduce these problems.

**Keyword:** Infertility, Demographic Data, Sexual Disorder

### **O<sub>nm</sub>-8: Recurrent Implantation Failure in IVF/ICSI Cycle**

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The success of assisted reproduction, although gradually increasing over the years, is still less than satisfactory. Many couples have benefited from this treatment; many have also been left frustrated following multiple failed attempts (Bulent Urman et al. 2005).

Repeated implantation failure (RIF), defined as three or more failed IVF attempts or failure of IVF after cumula-

tive transfer of more than 10 good quality embryos, represents one of the major challenges to assisted reproduction practice (Christophe Blockeel et al. 2008).

One of the prime factors implicated in the failure of assisted conception treatment is the inability of the embryo to implant into the endometrium. Several areas have been explored in attempts to improve implantation, including: the embryo transfer technique (Anderson et al. 2002; Pope et al. 2004); endometrial receptivity (Stern et al. 2003; Stephenson and Fluker 2000); the presence of hydrosalpinges and uterine fibroids (Barmat et al. 1999; Camus et al. 1999; Strandell et al. 1999; Hart et al. 2001; Check et al. 2002) and implantation potential of the embryo itself (Levitas et al. 2004; Caglar et al. 2005).

It is evident that the aetiology underlying recurrent implantation failure is complex that cannot be attributed to a single abnormality. In couples who repeatedly give rise to bad quality embryos, Preimplantation genetic diagnosis (PGD) may be undertaken to assess the ploidy status of individual embryos. The genetic analysis is performed on one or two blastomeres, by fluorescent in situ hybridization (FISH) for cytogenetic diagnosis, or polymerase chain reaction (PCR) for molecular diagnosis. Genetic analysis of the first or second polar body can be used to study maternal genetic contribution (Claire Basille et al. 2009). Such couples should be discouraged from undergoing further treatments if all embryos are aneuploid. When euploid embryos are found among the cohort, further treatment attempts may eventually be rewarded with a conception.

In couples who fail to conceive despite the transfer of good quality embryos, other factors that may impede implantation should be sought and corrected when identified. Normality of the uterine cavity should be confirmed with office hysteroscopy (Urman et al. 2005). Faulty embryo transfer technique should be rectified. Intratubal embryo transfer may be considered if the patient underwent previous difficult transcervical embryo Assisted hatching and blastocyst transfer can also be considered in patients with recurrent implantation failure. Screening for APA (Antiphospholipid antibody) is not routinely carried out, nor are empirical treatments such as aspirin, heparin, or leukocyte isoimmunization. co-cultures, these should be advised with caution. In couples with recurrent implantation failure with the transfer of good quality embryos, preimplantation genetic screening does not increase the implantation rates after IVF–intracytoplasmic sperm injection (Christophe Blockeel et al., 2008)

## **O<sub>nm</sub>-9: Pollution and Fertility**

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Air is our main contact with the environment in which we

find ourselves. Every day, we breathe in 15 kg of air; that's more than our daily food intake. Air, in cities and confined environments where houses are really close together, consists of particles of different sizes charged of chemical pollutants. The finer particles will come into contact with the alveoli and will allow some pollutants go directly into the bloodstream, resulting in diseases. Our environment is directly linked to emergence of diseases. Purifying the air in your house becomes vital. Thanks to new technological methods, it is possible to purify the air you breathe by reducing the number of micro-particles. Some other toxic chemical that can be found in the air we breathe at home are glycol ethers. They can cause infertility in men and women.

In recent years, numerous studies showed that exposure to environmental air pollutants affected reproductive functions and in particular, produced adverse effects on pregnancy outcomes.

Increased circulating Pb levels are also associated with adverse changes in sperm count, ejaculate volume, percentage of motile sperm, swimming velocity and morphology. Total sperm count and concentration were shown to decrease with increasing concentrations of blood Pb. Blood Pb levels are also inversely correlated with the percentage of live sperm found that even moderate exposure to Pb significantly reduced human semen quality but they did not find any conclusive evidence for Pb-related derangement of male reproductive endocrine function.

Interestingly, Pb accumulates in male reproductive organs; human testes and sperm contain numerous potassium channels through which metallic toxicants can enter into mature sperm (Pb can compete with, or even replace the Zn in human protamine at two different sites, so causing a conformational change in the protein. This interaction adversely affects sperm chromatin condensation, recently observed that sperm concentration was reduced by 49% in men with a blood Pb concentration >50 µg/dl; however, there was no indication of a linear trend of lower sperm concentration with increasing blood Pb values, and sperm chromatin deterioration was not correlated with blood Pb concentration. The comparative evaluation of sperm parameters, absorption markers and environmental concentrations indicates that Pb is probably causing the impaired spermatogenesis. Alteration of sperm function could be considered a precocious marker of detrimental toxicological effects. High rates of air pollution can reduce the number of boys born and may be linked to increased rates of miscarriage, the conference was told.

Pollution from air, food or water, or it might be radiation, thermal heat or chemical additives in food affects fertility. Men might improve their fertility by reducing how much pollution they breathe in. The dirtier the air, the lower a man's sperm count and the more sperm with fragmented DNA he produces. A study was conducted on thousands of patients in England discovered that women who regularly breathe in polluted space, have 24 percent smaller chance to get pregnant compared to

those who live in less polluted areas. Nitrogen dioxide, a toxin produced by the exhaust of cars and trucks and gas stove, have a negative impact on the opportunities of women to have babies.

### **O<sub>nm</sub>-10: Ginger Effects on Morning Sickness (Review)**

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**Introduction:** Mild-to-moderate nausea and/or vomiting affects up to 80% of all pregnant women, usually peaking by week 9 and subsiding by week 20. For 9% to 20% of women, nausea and vomiting of pregnancy persist longer; Nausea and vomiting are complex responses involving various neural pathways and motor responses to sensory stimuli.

**Definition:** Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment for various foods and beverages. Ginger has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds.<sup>1</sup> Characterized in traditional Chinese medicine as spicy and hot, ginger is claimed to warm the body and treat cold extremities, improve a weak and tardy pulse, address a pale complexion, and strengthen the body after blood loss.

**Mechanisms of Action:** The mechanism underlying ginger's anti-emetic activity is not clearly understood, but the aromatic, spasmolytic, carminative, and absorbent properties of ginger suggest it has direct effects on the gastrointestinal tract.

Ginger extracts exhibit inhibition of platelet aggregation and thromboxane synthesis in vitro, which has led to concerns ginger extracts may prolong bleeding; however, several European studies using ginger orally did not find any significant anticoagulant effects in vivo. The exact mechanism of action of ginger is thought to be a gastric effect, to increase tone and peristalsis due to anticholinergic and antiserotonin action.

**Dosage:** For most purposes a typical dose of ginger is 1-4 g daily, taken in divided doses. To prevent motion sickness, it is best to begin treatment 1-2 days before the scheduled trip and continue dosing throughout the duration of travel. For nausea and vomiting during pregnancy, ginger tea made from fresh ginger root, boiled and diluted to taste, appears to work best.

**Side Effects and Toxicity:** Ginger is on the U. S. Food and Drug Administration's GRAS (generally recognized as safe) list. The British Herbal Compendium documents no adverse effects of ginger.

Animal studies have reported both mutagenic and antimutagenic effects of isolated components of ginger,

and human studies have conflicting results regarding potential inhibition of platelet aggregation when ginger is consumed at high doses. One recent study examined pregnancy outcomes in 187 women known to have consumed ginger during the first trimester and found no statistically significant difference in major malformations, spontaneous abortion, and stillbirth rates between the ginger and the comparison group.

The FDA classifies ginger as "Generally Recognized as Safe," and the German Commission E monographs report no known side effects and no known drug/herb interactions.

Adverse effects after ingestion of ginger are uncommon, but they can include mild gastrointestinal effects such as heartburn, diarrhea, and irritation of the mouth. Because there is a possibility that ginger may affect fibrinolytic activity, it may be prudent for patients taking anticoagulants such as warfarin.

While data are insufficient to recommend ginger universally and there are concerns with product quality due to limited regulation of dietary supplements, ginger appears to be a fairly low-risk and effective treatment for nausea and vomiting associated with pregnancy. In low doses, this may be appropriate for patients not responding to traditional first-line therapies.

Animal studies demonstrate effects on the gastrointestinal tract, the cardiovascular system, on experimental pain and fever, antioxidative, antilipidemic and antitumor effects, as well as central and other effects. The most relevant human pharmacological studies require a confirmatory study to exclude interaction of ginger preparations with platelet aggregation. Pharmacokinetic data are only available for [6]-gingerol and zingiberene. Preclinical safety data do not rule out potential toxicity, which should be monitored especially following ginger consumption over longer periods.

**Final Comment:** Given that many antiemetic medications have the potential for sedation as a side effect, the use of ginger is a reasonable and safe alternative to treat pregnancy-induced nausea and vomiting.

### **O<sub>nm</sub>-11: Review of Multiple Pregnancies**

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In Multipregnancies, The careful control of Number of follicles in stimulation cycles and Number of embryos transferred in ART cycles is so important.

Just twin pregnancies are reason for racial death (12-15%).

The risk for CP in twin pregnancies is 8 times more and for Triple is 47 times more than simple ones.

Approximately all prenatal problems increase in multiple pregnancies and therefore this kind of pregnancies are considered high risk pregnancies.

One of the most issues in prenatal care in Multiple pregnancies is to detect the number of corions that its best detective time is in first trimester by about 100% accuracy. mono Corion pregnancies have more danger for Newborns for example 5 times increase in loss of fetus , 10 times increase in Low birth weight. Mono Corion twin Consequences are as follow:

Imbalanced Blood transfer between two fetus, mono amnions twin, death of one fetus in uterus (IUDF), TRAP twins and sucked twins.

### **O<sub>nm</sub>-12: Convictions of Health and Well - Being: Islam Women Living with Infertility in India**

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**Background:** The objectives of this study are aimed to find out the main relevant risk factors of female infertility and to meet the service needs of people who have problem associated with infertility. To reduce their feelings of social isolation on improved relationship with family and male partners. To promote physical, spiritual and mental health also knowledge and awareness of infertility among focus group.

**Materials and Methods:** This research is initiated for coping, health related quality of life and psychological well-being of women facing the problem of infertility. The research was organized between January to April, 2009 Nellore district rural area in southern part of India, with women facing infertility problems in their family setting. 40 Islam women ages spanned from 20-38 years (with an average of 26.8 years) who do not wish for mother hood via other routes expressed their concern to participate in this research. The period of no conceiving ranges from 2 – 10 years after marriage (with an average of 8.2 years). The subjects participated in this study expressed infertility is a problem of their social, psychological and stressful to marital life. Subjects income categories are below and just above the national average income. 21% of the participants have secondary education, 46% pre secondary education and the rest 33% are observant religious education. In a gesture to promote health perceptive of the focus group physical exercise, Meditation and counseling protocols were administered for 4 months. Pre and post test scores of cardio-vascular efficiency compared for physical health, Meditation for spiritual health, well-being, and anxiety and stress disorders. Causes of infertility, treatment priorities, knowledge and awareness through counseling.

**Results:** The over whelming success in an evidence through paired t test results indicated statistically significant increase in cardio-vascular efficiency, spiritual

health, well-being, anxiety and stress perceptive and positive marital relationship in infertile Islam marginalized female groups. Knowledge, awareness, causes and treatment priorities on infertility also found to be increased among the target group.

**Conclusion:** The findings supported that positive treatment protocols cause's significantly infertile females physical and psychological well-being perspectives. This findings lend support to the findings of Hoi-Yanchan, celia et al. (2005) and Geok-ling Lee et al. (2006) infertile people expressed psychological well-being, positive marital relationship and social support through the implementation of coping strategies.

### **O<sub>nm</sub>-13: Experiences of Oocyte Donation in Karolinska University Hospital.**

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Oocyte donation is allowed in Sweden according to a legislation accepted on the first of January 2003. Before then, Swedish woman had to seek these treatments outside of the country. The legislation had been under evaluation for several years so the need for this treatment option had accumulated and a large number of patients were on waiting list. There were also issues regarding criteria for treatment and economical compensation for the donors expenses, which needed clarification. This lead to a delay for an additional 6 months from the acceptance of the legislation.

There is a great demand for oocyte donation. It is very hard to specify how long the couples have to wait to get treated. This is due to the availability of donors. The waiting list depends resources allocated by the hospital and prioritation.

In our hospital the coordination is managed by midwives. Midwives is taking contact to both donors and recipients and also making the suggestions for donation. The doctor is shown the suggestions and is responsible for the treatments.

### **O<sub>nm</sub>-14: Cancer, Infertility and Supportive Role of Nurse**

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### **O<sub>nm</sub>-15: Reproductive Factors and Primary Infertility in Patients with Breast Cancer**

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### **O<sub>nm</sub>-16: Ethics Issues in Assisted Reproductive Technology**

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### **O<sub>nm</sub>-17: Quality of Semen; Roles of Dietary Factors: A Review of Literature**

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**Background:** Infertility is an unfavorable issue in reproductive health worldwide. At least one in six couples refers to infertility clinic. Various factors including weight, diet, genetic factors, smoking and exposure to extreme heat, environmental pollutant, infections and medical disorders are suggested to be involved with infertility disorder.

**Materials and Methods:** Through a systematic review of literature using Medline and Pubmed from May 2009 onward, overall 6 case-control articles had inclusion criteria and were reviewed accordingly.

**Results:** There are several lifestyle elements including dietary factors are reported to be involved with infertility problem. Antioxidants decrease sperm damage especially in men with DNA fragment and lipid peroxides. Oxidative stress can be resulted in poor semen quality by selenium and decreasing expression of HSP70 protein which is play a role in germ cell differentiation and spermatogenesis and 39% of men with abnormal semen have had low levels of Cobalamin. Also 62% of men with more than 24% DNA fragment index (DFI) who had received antioxidant for 3 month were successful in having baby, whereas the corresponding rate was 37% in the control group. Decreased alcohol and coffee consumption as well as smoking cessation programs in couples with normal sexual activity have improved semen quality up to 76%. Men with sperm count more than 20 million have had more carbohydrate, fibers, foliate, Vit C, licopen intakes and less protein and fat in their regimen. More than one-third (35.0%) of infertile men who routinely used sea foods, had an elevated mercury blood level. Low fruits and vegetables intake were the significant risk factors associated with oligospermia.

**Conclusion:** Quality of semen decreases with diet full of dairy and meat which could be in part due to existence of lipophilic materials like Xenoestrogens and anabolic

steroids.

**Keyword:** Infertility, Semen Quality, Dietary

### **O<sub>nm</sub>-18: The Causal for Repeated Implantation Failures**

Toousi M

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**Background:** In spite of the great deal of research in assisted reproductive techniques, more than 80% of transferred embryos by IVF/ICSI methods fail to be implanted. The causes for repeated implantation failures (RIF) may be reduced endometrial receptivity or other various uterine pathologies, such as thin endometrium, altered expression of adhesive molecules or immunological factors; whereas genetic abnormalities of male or female individuals, sperm defects, embryonic aneuploidy or zona hardening are other etiologies for implantation failures. Clinically, endometriosis, polycystic ovaries and hydrosalpinx may decrease implantation following embryo transfer due to dual disorders in the quality of embryos or endometrium.

**Material and Methods:** This article presents the result of a systematic review about Repeated Implantation Failures.

**Results:** Probable causes and methods of evaluation for RIF patients have been reviewed and the suggested methods for their treatment.

**Conclusion:** In this study, probable causes and methods of evaluation for RIF patients have been reviewed and the suggested methods for their treatment, including myomectomy, endometrial stimulation, immunotherapy, hysteroscopy, preimplantation genetic screening (PGS), assisted hatching, zygote intra-fallopian transfer (ZIFT), co-culture, blastocyst transfer, cytoplasm transfer, tailoring stimulation protocols, intracytoplasmic sperm injection (ICSI) and salpingectomy for hydrosalpinges have been discussed.

**Keywords:** Repeated Implantation Failure, Embryo Transfer, Endometrial

### **O<sub>nm</sub>-19: The Role of Cord Blood Preservation in Cell Therapy**

Zarabi M.

Royan Cord Blood Bank, Tehran, Iran

During pregnancy, the placenta delivers "cord blood" to the baby through the umbilical cord serving as a lifeline of nourishment from the mother to baby. At birth, "cord blood" remains in the umbilical cord and placenta and until recently, had typically been discarded. The tragedy of this practice is that "cord blood" contains very special cells called "stem cells". Recent advances in medical research, have allowed us to identify stem cells as the building blocks of every type of cell in our bodies.

In light of these and other recent advances in medical research, people have started saving or "banking" their baby's cord blood.

Cord blood can be collected both at vaginal delivery and in connection with Caesarean section. Cord blood is collected from the remains of the umbilical cord. Remains that would have otherwise been discarded immediately after birth. It is collected with a syringe by puncturing the umbilical vein immediately after the umbilical cord has been clamped, cut, and cleaned with antiseptic solution. Cord blood is a very rich source of stem cells. This very rich source of stem cells must be collected at birth or it is lost forever.

If your child or someone else in your family develops a life-threatening and/or debilitating disease or condition, they may be considered for a stem cell transplant. Banking your baby's cord blood ensures that you will have your family's stem cells readily available as a life-saving resource.

Umbilical cord blood stem cells are the "most immature," available form of stem cells that are genetically unique to your baby and to your entire family. Cord blood derived stem cells are also free of controversy associated with embryonic stem cells, another type of stem cells. Storing these cells essentially stops the clock and prevents aging and damage that would normally occur to cells as they age. A third type of stem cells is adult stem cells, such as those found in bone marrow. Bone marrow stem cells are "older" stem cells, and are less desirable as a treatment option because they are 1) less readily available as they more difficult to harvest (involves putting the donor's life at risk), 2) less suitable for other family members (degree of match has to be greater because these cells are older and 3) are associated with poorer treatment outcomes.

**Medical Uses:** The following conditions are currently being treated with stem cells. Cord Blood derived stem cells are an area of intense ongoing research. The list of diseases treated with stem cells is growing daily. Any disease which requires the regeneration of tissue (due to injury or disease) is a potential candidate for stem cell therapy.

**Hemoglobinopathies/Blood Disorders:** Sickle-cell anemia,  $\beta$  thalassemia.

**Inborn Errors of Metabolism:** Adrenoleukodystrophy, Batten disease, Gunther disease, Hunter syndrome, Hurler syndrome, Lesch-Nyhan disease, Tay-Sachs disease

**Immunodeficiencies:** Chronic granulomatous disease, Common variable immune deficiency (CVID), Omenn's syndrome, Severe combined immune deficiency (SCID and SCID-ADA), Reticular dysgenesis, Thymic dysplasia, Wiskott-Aldrich syndrome, X-linked lymphoproliferative disease, Bare lymphocyte syndrome (MHC class II deficiency), Leukocyte adhesion deficiency

**Other diseases:** Evans syndrome, Familial erythrophagocytic hemophagocytic, Langerhans cell histiocytosis, Osteopetrosis.

**Heart regeneration** to repair injury due to heart attack.

**Brain regeneration** to repair injury, due to stroke, heart attack, Alzheimer's

## Poster Presentations

### **P<sub>nm</sub>-1: Progress of Integrative Chinese and Western Medicine in Treating Polycystic Ovarian Syndrome Caused Infertility**

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Polycystic ovarian syndrome (PCOS) is one of the most popular diseases that cause menstrual dysfunction and infertility in women. The present paper is a brief retrospective on the progress in treatment of PCOS caused infertility with integrative Chinese and Western medicine (ICWM). It can be seen from these materials that using traditional Chinese medicine (TCM) recipes formulated by Shen-replenishing herbs or acupuncture to reinforce Gan-Shen, regulate Chong-Ren Channels in treating PCOS, stable clinical efficacy could be obtained, with less adverse reaction, though the effect initiated somewhat late. Whereas, when Shen-replenishing recipe and acupuncture are combined with hormone or ovulation promoting drugs of Western medicine, the above-mentioned shortcomings would be overcome. So, this combined therapy is frequently used in clinical practice.

### **P<sub>nm</sub>-2: Anxiety Level during Pregnancy after ART and Relation with Duration of Infertility**

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1. Student of M.Sc Midwifery in IUMS
2. Senior Lecture, School of Nursing and Midwifery, IUMS
3. Member of Nursing Research Center, School of Nursing and Midwifery, IUMS
4. Senior Lecture, School of Management, IUMS

**Background:** Pregnancy after assisted reproduction technology is the one of risk factors that can result in anxiety. Many studies showed that for up to 20% of infertile women, the emotional burden of infertility persist even after successful treatment. This study aims to determine anxiety level and its relation with duration of infertility in ARTs pregnant women undergoing sample infertility centers in Tehran.

**Materials and Methods:** In this cross sectional study, 100 ARTs pregnant women were participated. Data was collected by Beck Anxiety Inventory (BDI) and personality/demographic questionnaires. Data analysis was performed with SPSS.V.14.

**Results:** Mean age was 33.7±6.81 and mean of married duration, gestational age and infertility duration was 8.70±5.18, 20.18±10.8 and 7.37±6.81 respectively. Of 45 pregnant women in first trimester, 28/9% (n=13) have moderate and sever anxiety. Of 20 pregnant women in second trimester, 35% (n=7) have moderate and sever anxiety and finally, of 20 pregnant women in third trimester, 40% (n=14) have moderate and sever anxiety. There

is a significant relation between anxiety level and duration of infertility ( $p=0.03$ ).

**Conclusion:** Careful consideration and psychological consultation for ARTs pregnant women is necessary to detect high-risk pregnant and reduce the anxiety and poor pregnancy outcomes.

**Keywords:** Anxiety Level, Duration of Infertility, Assisted Reproductive Technology

### **P<sub>nm</sub>-3: Abortion and Breast Cancer Risk: A Case-Control Study in Tehran, 2005**

Dadashi MRO\*, Shahporian L, Hosseini Z, Motamednia L

Iran University, Tehran, Iran

**Background:** Large part of research related to reproductive factors in breast cancer risk before and after menopause, a study case - control is that it factors in deliberate and spontaneous abortion in the two groups of women with breast cancer (cases) and non-infected women (control group) study, described odds ratio in women, breast cancer was measured. In this study, after recognizing the above factors in breast cancer risk in their separation, before menopause and after menopause were considered.

**Materials and Methods:** 500 cases referred to shohadaye Tajrish Hospital and cancer Institute of Imam Khomeini hospital that they 250 premenopausal and 250 postmenopausal breast cancer patients were a select group method and continuous participation in the research was. The control group was composed of 500 persons during the study as visitor centers or with or referred to in the vicinity of group homes, women had lived in Tehran and sampling were selected in available or easy.

**Results:** Employing logistic regression analysis and calculated the risk ratio findings showed: history of 2 spontaneous abortion ( $p=0.043$  and  $OR=2.38$ ) in total breast cancer wasn't associated but separated before and after menopause correlation. Induced abortion in total ( $p=0.018$  and  $OR=1.57$ ) And at times before the menopause ( $p=0.018$  and  $OR=2.03$ ) were associated with breast cancer.

**Conclusion:** According to the findings can be concluded that abortion is not itself a significant risk factor for breast cancer.

**Keywords:** Breast Cancer, Spontaneous Abortion, Induced Abortion

### **P<sub>nm</sub>-4: Role of Hormonal Factors and Use of Contraceptive Pills on Breast Cancer**

Dadashi MRO\*, Shahporian L, Hosseini Z, Motamednia L

Iran University, Tehran, Iran

**Background:** Large part of research related to re-

productive factors in breast cancer risk before and after menopause, a study case - control is that it factors in deliberate and spontaneous abortion in the two groups of women with breast cancer (cases) and non-infected women (control group) study, described odds ratio in women, breast cancer was measured. In this study, after recognizing the above factors in breast cancer risk in their separation, before menopause and after menopause were considered

**Materials and Methods:** 500 cases referred to shohadaye Tajrish Hospital and cancer Institute of Imam Khomeini hospital that they 250 premenopausal and 250 postmenopausal breast cancer patients were a select group method and continuous participation in the research was. The control group was composed of 500 persons during the study as visitor centers or with or referred to in the vicinity of group homes, women had lived in Tehran and sampling were selected in available or easy.

**Results:** Employing logistic regression analysis and calculated the risk ratio findings showed that: The use of hormonal methods of contraception ( $p=0.036$ ,  $OR=1.39$ ), more than 25 years of age at the time of starting the tablets ( $p=0.052$ ,  $OR=2.99$ ) and less than 5 years from the last tablet from the time of diagnosis ( $OR=0.000$ ,  $p=3.28$ ) with breast cancer are connected.

**Conclusion:** According to the findings can be concluded that taking contraceptive pills is own weak risk factor for breast cancer.

**Keywords:** Breast Cancer, Hormonal Factors, Contraceptive Pills

#### **P<sub>nm</sub>-5: Evaluation of Infertility Menopausal Women's Attitudes towards Menopause in Shiraz**

Ghazanfarpour M\*, Goudarzi M

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**Background:** This study was conducted to evaluate the attitudes of infertile menopausal women towards the menopause and its relation with demographic characteristics.

**Materials and Methods:** This was a cross-sectional study that evaluated 189 infertile menopausal women attending primary health care centers in Shiraz. Five health centers in different parts of the city were chosen and the participants were selected from these women attending the above-mentioned health care centers randomly. Then, they were interviewed and a questionnaire was filled in.

**Results:** The mean age of the participants was  $54.9(\pm 5.5)$  years. 160(42.3%) were illiterate and 9(2.4%) had a college degree. Seventy (18.5%) of them were smoker. The mean and standard deviation of the total score of attitude was  $102.7\pm 11.8$  (Rang: 71-135). As statistically significant relation was observed between

during of infertility and total score of attitude in multiple regression analysis.

**Conclusion:** According to the results of the study, there are some differences various between infertility women and these differences lead to different attitudes among in women. Infertility is very important in attitudes towards menopause. Therefore, it is very important to make some plans for evaluating infertility menopausal and controlling them.

**Keywords:** Menopause, Attitudes, Infertility

#### **P<sub>nm</sub>-6: Physical Activity and Infertility**

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#### **P<sub>nm</sub>-7: Effect of Folic Acid and Zinc Sulfate Prescription on Functional Parametrs of Sperm in Infertile Men**

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Torbat Heidariyeh Medical University, Mashhad, Iran

#### **P<sub>nm</sub>-8: Infertility Prevention and Reproductive Preserve in Women Following Uterine Fibromas**

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#### **P<sub>nm</sub>-9: Infertile People's Attitude towards the Role of Nutrition in Infertility**

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2. Nutrition Department, Research Center of Nutrition Science, Helath and Nutrition University, Tabriz, Iran

#### **P<sub>nm</sub>-10: Assessment of Common Reasons for Infertility in Men and Women in Neishaboor in 1388-1389**

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#### **P<sub>nm</sub>-11: Vitex Function in Infertility**

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### **P<sub>nm</sub>-12: Celiac Disease and Its Effect on Human Reproduction**

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**Background:** Celiac disease is an intestinal inflammatory disease that is triggered by gluten in the diet. Patients present with a wide array of symptoms due to malabsorption that include diarrhea, abdominal pain, and bloating and weight loss.

**Materials and Methods:** In women, this disease may have implications on menstrual and reproductive health. The symptom complex includes delayed menarche, early menopause, secondary amenorrhea, infertility, recurrent miscarriages and intrauterine growth restriction.

**Results:** These women benefit from early diagnosis and treatment. Therefore, celiac disease should be considered and screening tests performed on women presenting with menstrual and reproductive problems and treated accordingly.

**Conclusion:** The objective of this article is to review the current literature on celiac disease and its association with the above-mentioned disorders.

**Keywords:** Celiac, Human Reproduction

### **P<sub>nm</sub>-13: Young People and Infertility Related to Airpollution**

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### **P<sub>nm</sub>-14: Evaluation of Pregnancy Consequences Related to Frequent Sonographies**

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### **P<sub>nm</sub>-15: Exercise-Related Female Reproductive Dysfunction**

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Midwifery Department, Shiraz University, Shiraz, Iran

**Background:** Participation of adolescents and young women in strenuous sports activity may lead to various metabolic and psychological derangements of clinical relevance to the endocrinologist.

**Materials and Methods:** The most common manifestations encountered in practice are primary and secondary

amenorrhea, reduced bone mineral density and eating disorders. The occurrence of all three together has been named "the athletic triad". The underlying hormonal drivers that lead to some of these manifestations are the reduced leptin level as well as the persistent low grade stress response commonly observed in such females.

**Results:** "Exercise-related female reproductive dysfunction" (ERFRD), can possibly include short-term (infertility) and long-term (osteoporosis) consequences. Functional hypothalamic amenorrhea, a manifestation of ERFRD in adolescence, is an integrated response to the combination of excessive physical and emotional stress, exercise, and/or reduced food intake characterized by decreased endogenous GnRH secretion.

**Conclusion:** The primary aim of treating these athletes should be the prevention of the development of any component of the triad as well as the whole complex by educating athletes, trainers, parents and health care professionals about proper nutrition and safe training. The long term prognosis is good. However, significant long term morbidity may affect these young women later in life.

**Keywords:** Exercise, Female Reproductive Dysfunction

### **P<sub>nm</sub>-16: A Peer Model Experience for University Students' Health Promotion**

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4. Students and Cultural Deputy, Qazvin Medical Science University, Tehran, Iran

**Background:** A peer group is a group with the same age, social status and interests that its characteristics lead to consider peer education as an effective behavioral change strategy in reproductive health worldwide. Aim to assessment of peer interventional health programs in students groups this study has been designed and conducted based on peer participatory approach.

**Materials and Methods:** This interventional study has been conducted in a medical science university through stakeholders' partnership and selection of 24 volunteer students according to their knowledge, interest, communication skills, and etc. Then capacity building of them was performed through holding a highly interactive Reproductive Health Course contained the topics, Marital Health, Pregnancy and consequence of illegal Abortion, Family planning, STI/AIDS, Communication and Counseling skills. Trained peer educators have introduced to other student and present education and counseling formally and informally. A post interventional study has

been conducted after 9 month in order to find its effectiveness.

**Results:** In this way, peer education and counseling was evaluated for assess its effectiveness. As a result, %64/7 of students was agreed with peer education and they were feeling empathy due to same condition of peers (%60) especially in sensitive topics. %55.3 of them felt need to existence of peer educator core in the university. According to students' opinion, we conclude Peer education is effective strategy for reproductive health promotion and reinforces positive behaviors in youth.

**Conclusion:** Universities are appropriate real world for experience a friendly youth program and then disseminate it to other young communities. There seems peer education is effective strategy for Reproductive Health promotion and reinforce positive behaviors in youth.

**Keywords:** Peer, Health, University Students

### **P<sub>nm</sub>-17: Nursing in Hyperstimulation**

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### **P<sub>nm</sub>-18: Role of Micronutrients in the Periconceptional Period**

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**Background:** Micronutrient deficiencies have been associated with significantly high reproductive risks, ranging from infertility to fetal structural defects and long-term diseases.

**Materials and Methods:** In this review we focus on the reproductive risks related to some micronutrients during the periconceptional period, a critical step in determining fetal development and health due to the potential onset of several disorders.

**Results:** Fertility, conception, implantation, fetal organogenesis and placentation are the critical stages potentially affected by nutrition during the periconceptional period. Reactive oxygen species (ROS) and total homocysteine (tHcy) plasma levels are factors involved in the respective mechanisms. The preconceptional period is particularly important since it affects both fertility and the early stages of gestation. Micronutrients' dietary intake and maternal status affect the different phases of the onset and development of pregnancy as well as of the conceptus.

**Conclusion:** Although human studies are scarce, and conclusive evidence is provided solely for periconceptional folate and prevention of neural tube defects (NTDs), the overall data indicates that micronutrients may affects fertility, embryogenesis and placentation, and the prophylactic use of some micronutrients may be useful in preventing several adverse pregnancy out-

comes. Efforts to increase awareness of a healthy diet should be strengthened not only throughout pregnancy but also before.

**Keywords:** Micronutrients, Periconceptional Period, Infertility

### **P<sub>nm</sub>-19: Ovarian Function and Obesity--Inter-relationship, Impact on Women's Reproductive Lifespan and Treatment Options**

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**Background:** Insulin resistance (IR) is a consequence of obesity, and in women it is often inextricably linked with ovarian function leading to clinical reproductive manifestations such as early menarche onset, subfertility and polycystic ovary syndrome (PCOS).

**Materials and Methods:** Likewise, the dramatic fall in oestrogen production after menopause may contribute to weight gain and changes in adipose tissue distribution.

**Results:** Overall, women who are obese, especially those with reproductive complications including PCOS, have been identified as specific high risk subgroups for further progression through to prediabetes, type 2 diabetes mellitus (T2DM) and potentially cardiovascular disease (CVD).

**Conclusion:** This review focuses on the interrelationship between the ovarian function and obesity as well as its treatment strategies.

**Keywords:** PCO, Obesity, Women's Reproductive

### **P<sub>nm</sub>-20: Sexual Disorders in Infertile Couples**

**Safdar M\*, Azima S**

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**Background:** Sexuality and the desire for a child are strongly interconnected. The same applies to sexual disorders and the unfulfilled desire for a child. Aim: This article indicates the relations between sexual disorders and the unfulfilled desire for a child and outlines the potential effects of diagnostics and treatment in the context of reproductive medicine on the couples' sexuality.

**Materials and Methods:** This research drive was undertaken in well-established medical and psychological literature database with the keywords "infertile" or "infertility" and "sexual dysfunction" or "sexual satisfaction."

**Results:** Sexual dysfunctions (of organic or of psychic origin) as a cause of involuntary childlessness are relatively unusual. By contrast (temporary) sexual disorders resulting from diagnosis and medical therapy are common in couples with fertility problems, with women more frequently affected than men.

**Conclusion:** Counseling for couples with the unfulfilled desire for a child should invariably include explicit and

appropriately tactful reference to sexuality and (functional) sexual disorders.

**Keywords:** Sexual Disorders, Infertility

### **P<sub>nm</sub>-21: Knowledge Assessment of Women with Primary Infertility about Effective Factors of Onset or Continues Infertility**

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### **P<sub>nm</sub>-22: The Role of Sex Differences in Mental Aspect of Infertility**

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Infertility is one of the couple's problems in different society. Based on the researches done, the rate of infertility is almost 12 to 20 percent. Studies considered infertility as a source of mental disorders. It has not only physiological aspects but also psychological aspects. The results of the studies done by mental health specialists, psychologist, have shown that infertile people are affected more by chronic stress and they are at the risk of diseases like depression, anxiety, low self-esteem and dissatisfaction. In general experiencing stressful event causes. The decrease of person's control in life and makes the couple irritable and unstable. It also results in aggressive modes, feeling of disability, disappointment, uselessness, embarrassment and anxiety. Infertility makes sexual relations undesirable. The results obtained from Mahlsdet's study (1987) done with 63 infertile female and 37 infertile male showed that 96% felt disappointment, 81% unenjoyment, 82% irritability and 65% anger, sex is one of the factors that effects the way people react toward infertility. A lot of studies were done in this regard. The results taken from most of them have shown that the experience of infertility is more stressful for women than the men. The infertile women have had more psychological problems than men and infertility has more negative meaning for women than for men Shaver et al. (1992), Gril (1991) have done studies on fertility and concluded that women in comparison with men enjoy. 1. Life confidentiality 2. High depression 3. Low life satisfaction 4. Lack of child as an unacceptable problem 5. Considering infertility as their fault. In terms of depression and anxiety, Domar and et al. (2000) have done some researches. They indicated that the degree of depression and anxiety is higher in women than in men. Newton et al. concluded that shared life for women is less enjoyable than men. What we should know is that the difference in sex and its effect on infertility is more important than the problem

person. The role of sex and sexual identity are of great importance in infertility. What we should consider more is the psychosocial aspects of infertility.

What we should know is that the difference in sex and its effect on infertility is more important than the problem person. The role of sex and sexual identity are of great importance in infertility. What we should consider more is the psychosocial aspects of infertility.

**Keywords:** Sex Differences, Mental Aspect, Infertility

### **P<sub>nm</sub>-23: Anxiety during Pregnancy and Fetal Attachment after *In vitro* Fertilization Conception**

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**Background:** Attachment style is the pattern of an individual in forming relationships with others, which is believed to be determined in the early years of life. Maternal-fetal attachment (MFA) is a term used to describe the relationship between a pregnant woman and her fetus. Qualitative descriptions of maternal attitudes and adaptation to pregnancy indicate that MFA is based on cognitive representations of the fetus. These may include imagined scenarios between mother and child, as well as a mother's attribution of physical and emotional characteristics to the fetus. Mental health during pregnancy affects on fetus growing and baby's future adaptation. Therefore using different ways to promoting maternal mental health can decrease maternal-child problems. Many intervention programs were designed to promote maternal mental health. One of these interventions is improving relationship between mother and child. The aim of this study was to compare couples who had conceived by *in vitro* fertilization (IVF) with matched controls for the prevalence of anxiety and quality of attachment to the baby during pregnancy.

**Materials and Methods:** This article presents the result of a systematic review about the effect of behavioral attachment on maternal after *in vitro* fertilization conception.

**Results:** The results of this study demonstrate that, despite the finding of no group differences in state and trait anxiety using the Spielberger, the IVF mothers were significantly more anxious about the well-being of their unborn babies and about damage to the babies during childbirth (associated with more negative feelings about the birth and a tendency to a higher tolerance for medical interventions during the birth).

**Conclusion:** Education MFA behaviors during pregnancy can increase prenatal attachment and maternal mental health. So we can promote maternal mental health with education these simple behaviors *in vitro* fertilization conception.

**Keywords:** IVF, Parental Anxiety and Attachment, Pregnancy, Sign Specific Measures, Psychological Support

## **Pre-Congress Courses and Workshops**

### **Application of Color Doppler Sonography in Fetal Assessment**

Doppler ultrasound has important screening and diagnostic capabilities. In addition, Doppler ultrasound has an important contribution to make to the surveillance of the fetus compromised by intrauterine growth restriction (IUGR). This study can be used in a complementary fashion to the traditional biophysical profile and non stress test. Doppler studies have been subjected to a number of important randomized controlled trials, thereby placing these techniques within the realm of evidence-based medicine. The advent of color and pulsed Doppler techniques has permitted the use of Doppler methods to assess fetal structural abnormalities and complex disease processes involving fetal hemolytic anemia, twin-twin transfusion, and non immune hydrops. As a simple, noninvasive method of determining blood flow velocities; Doppler has been used in obstetrics diagnosis for more than 20 years. Doppler ultrasound of the fetal vascular system is an important adjunct for the evaluation of high-risk pregnancies in obstetrics and prenatal medicine. The selective use of fetal Doppler leads to significant reduction in prenatal mortality and morbidity.

This workshop has been organized by **Department of Reproductive Imaging- Royan Institute** to provide an overview of the role of Doppler ultrasound in prenatal fetal assessment.

**Chairman:**

- Babak Sanei, M.D., Radiologist

**Co-Chair:**

- Firoozeh Ahmadi, M.D., Radiologist

**Executive Manager:**

- Fatemeh Zafarani, B.Sc.

## **Clinical Application of DNA Fragmentation Assessment in Male Infertility Diagnosis**

Disorders of the male reproductive system have become an important public health issue as they can cause infertility, miscarriages and abnormal outcomes in the offspring. It is well recognized that spermatozoa from infertile men often have multiple structural and functional defects. Several recent studies have shown that sperm DNA fragmentation is a completely independent variable with little or nothing to do with the parameters that we measure on the routine semen analysis. It seems that the degree of DNA fragmentation correlates highly with the inability of the sperm to initiate a birth, regardless of the technology used to fertilize the egg.

In this workshop, some methods of chromatin damage assessment such as: AOT (Acridine Orange Test), SCSA (Sperm Chromatin Structure Assay), SCD (Sperm Chromatin Dispersion Test) and CMA3 (Chromomycin A3) will be trained and their clinical applications will be discussed. It may be useful for physicians and biologists who wish to learn more about the clinical aspects of DNA fragmentation assessment.

### **Chairman:**

- Mohammad Ali Sadighi Gilani M.D., Urologist

### **Executive Manager:**

- Marjan Sabaghian Ph.D.

## 3D XI of Scrotal Mass Lesion

Ultrasound plays an important role in the diagnostic workup of scrotal diseases. Ultrasound of the scrotum is the primary imaging method used to evaluate disorders of the testicles and surrounding tissues. It uses harmless, high-frequency sound waves to form an image. The sound waves are reflected by scrotal tissue to form a picture of internal structures. It is not invasive and involves no radiation. It is used when a patient has acute pain in the scrotum. Some of the problems for which the use of scrotal ultrasound is valuable include an absent or undescended testicle, an inflammation problem, testicular torsion, a fluid collection, abnormal blood vessels, or a mass (lump or tumor). It can differentiate a testicular mass from an extratesticular mass and determine whether the mass is cystic, solid, or complex. Three-dimensional extended imaging (3D XI), which has become available with advances in 3D US, is a powerful processing technique that gives the ability to view an obtained volume with different viewing methods.

This workshop has been organized by **Department of Reproductive Imaging-Royan Institute** to provide an overview of the role of 3D XI as a diagnostic procedure in scrotal mass lesion.

**The Workshop includes two parts:**

- Lecture on “Training curriculum in the practice and interpretation of 3D US imaging”.
- Procedure of 3D XI of scrotal mass lesion

**Chairman:**

- Salah Elwagdy, Professor of Genito-Urinary Imaging

**Co-chair:**

- Ahmad Vosough Taqi Dizaj, M.D., Radiologist

**Executive Manager:**

- Fatemeh Zafarani, B.Sc.

## Office Hysteroscopy

Nowadays hysteroscopy has considerably evolved in favour of patient comfort. Thanks to smaller instruments, but perhaps more importantly introduction of the vaginoscopic approach, the use of speculum and tenaculum are no longer necessary. Because performing a vaginoscopic biopsy or a small surgical intervention is the gold standard during evaluation of the uterine cavity, removal of certain intrauterine adhesions or polyps can easily be performed without anesthesia and in an office setting

This workshop is designed to introduce:

- Office hysteroscopy
- Main indications for hysteroscopy
- Performing certain hysteroscopic operations

**Chairman:**

- Ensieh Sh. Tehraninejad, M.D., Gynecologist

**Executive Manager:**

- Firoozeh Ghaffari, M.D., Gynecologist

## Oocyte, Embryo and Ovarian Tissue Staining Techniques

Assessing the quality and effect of different treatments on oocyte, embryo and ovarian tissue reveals essential information which could greatly affect and predict the outcome of IVF, IVM, Ovarian tissue transplantation, Drug treatments and many other different procedures.

This workshop focuses and enables a hands-on experience on various staining techniques to assess the effect of different treatments on oocyte, embryo and ovarian tissue.

**Chairman:**

- Hossein Imani, Ph.D.

**Executive Manager:**

- Rouhollah Fathi, M.Sc.

## **Seminal Vesicle Aspiration (SV Aspiration)**

SV aspiration is a potential test for the diagnosis of partial EDO. This procedure confirms the presence of intact spermatogenesis, and rules out more proximal obstruction, obviating the need for testicular biopsy. In addition, SV aspiration can be performed without anesthesia or X-ray in an office setting, and use of dye or contrast medium is not necessary.

### **Scientific Managers:**

- Gulgun Engin, M.D., Radiologist
- Mohammad Ali Sadighi Gilani, M.D., Urologist

### **Executive Manager:**

- Leila Daliri, M.Sc.

## Pre-Congress Course on Implantation

Implantation is an important step in establishing a pregnancy and is of major concern in the management of infertility. Failure at this step greatly limits the success of all approach to infertility treatments as well as assisted reproductive technology (ART). Immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss. Most of the recent investigations suggest differences in the expression of some immune cells and molecules in women with recurrent miscarriage.

Therefore, understanding the roles of local and systemic immune factors, cytokines, growth factors, adhesion molecules and other matrix-associated proteins in uterine receptivity for implantation is necessary to develop approaches to enhance reproductive health and fertility in humans. Here in this panel we aim to discuss the latest finding related to physiological and immune factors regarding to implantation. Then, we'll talk about the most recent approach to implantation failure including four lectures in this regards;

1. Introduction to Implantation.
2. Immunology and physiology of implantation.
3. Immunotherapy in the patient with implantation failure.
4. Clinical aspect of recurrent implantation failure.

And finally, we'll have three hours open discussion in the implantation panel.

**Chairman:**

- Mahnaz Ashrafi, M.D. Gynecologist

**Executive Manager:**

- Reza Aflatoonian M.D., Ph.D.

## Authors' Index

### A

Abasi P (P-49, P-50)  
Abavisani A (P-1)  
Abbasi Z (P-2)  
Abbasy E (P-35)  
Abdi H (P-49, P-50)  
Abdoli A (O-1, Abdoli A (P-3)  
Abdolvahhabi MA (O-9)  
Abedelahi A (P-21)  
Abolhasani F (O-9)  
Abousaoud W (I-30)  
Abousetta A (P-46)  
Absalan F (O-38, O-39)  
Abu Marar E (O-25)  
Acharya N (O-48)  
Achermann JC (O-43)  
Adib M (P-12)  
Aflatoonian A (I-29, O-26, O-47, P-56)  
Aflatoonian R (I-30)  
Afrough M (P-22)  
Aggarwal A (P-4)  
Agha Hosseini M (O-27, P-45)  
Ahangarpour A (P-65)  
Ahmadi F (Onm-1)  
Ahmadi G (Onm-2)  
Ahmadi Jalali Moghaddam M (P-23)  
Ahmadi R (P-7)  
Ahmadian S (O-9)  
Aitken RJ (O-9)  
Ajonuma LC (O-28)  
Akbari Asbagh F (P-52, P-53, P-54)  
Akhlaghpoor Sh (I-55)  
Akhondi MA (P-39, I-31, O-7)  
Aleyasin A (O-27, P-45)  
Al-Hasani S (I-18, I-3, I-4, O-25)  
Alian Moghaddam N (O-20)  
Al-Inany H (P-46)  
Alizadeh AR (O-4)  
Alizadeh F (P-47)  
Allameh AA (P-21)  
Allan HT (O-18, P-40)  
Allen P (O-6)  
Amidi F (O-41)  
Amini L (P-38)  
Amiraslani Z (Pnm-6)  
Amiri Z (P-14)  
Amirzargar A (P-65)  
Amirzargar MA (P-5)  
Ankolkar M (I-50)  
Ansary A (O-7)  
Aplin JD (I-5, I-6, I-7)  
Ardalan A (O-24)  
Ardekani AM (P-48)  
Asadi S (P-39, P-61)  
Asgari S (P-39)  
Ashrafi M (I-31, O-34)  
Asnaashari F (Onm-6)  
Atwood CS (P-24)  
avies-South D (O-6)  
Aytac O (I-2)

Azab H (P-46)  
Azad F (P-6)  
Azadbakht M (P-28)  
Azargoon A (O-29)  
Azarn M (P-27)  
Azarnia M (P-23, P-36)  
Azima S (P-13, P-16, P-62, Pnm-1, Pnm-12, Pnm-15,  
Pnm-18, Pnm-19, Pnm-20)  
Azizi M (P-20)  
Azodi P (P-10)

### B

Bagery Lankarani N (P-20)  
Baghbanzadeh A (P-1)  
Bagheri A (O-19)  
Bahadori MH (O-13, P-23)  
Bahman Abadi A (O-34)  
Bahmanpour S (P-26, P-6)  
Bakhshi S (O-7)  
Balali Sh (P-7)  
Balasinor NH (I-1)  
Balbach SB (I-8)  
Barati F (P-2, P-22)  
Barbarestani M (O-9)  
Baruffi RLR (O-15)  
Bashamboo A (O-43)  
Bazot M (I-59)  
Bazrafshan E (Pnm-1)  
Berisha B (O-46)  
Bjurensten K (Onm-3)  
Boiani M (I-8)  
Bonsel GJ (O-16)  
Bourlet T (O-2)  
Brauner R (O-43)  
Brosens JJ (I-32, I-33, I-34)  
Burgos J (P-70)

### C

Calaf J (O-32)  
Cameron ST (O-30)  
Cavalcante LF (P-32)  
Celtik M (I-2)  
Chaharsoughi SA (P-48)  
Chamani-Tabriz L (P-39)  
Chan HC (O-28)  
Chandrasekar M (O-3)  
Chavoshzadeh AF (P-35)  
Chedumala R (O-23)  
Chehrazi M (O-4)  
Chehreh H (Pnm-2)  
Chen HJ (P-29)  
Chien CH (O-31)  
Chien EJ (O-31)  
Chung YW (O-28)  
Corcostegui B (P-70)  
Crisol L (P-70)  
Crosignani PG (I-35, I-36, I-37)

### D

D'Souza R (I-1)  
Dadashi MRO (Pnm-3, Pnm-4)  
Dadashi RO (Onm-4)  
Dalimi A (O-1, P-3)

Darai E (I-59)  
Darbandy M (P-49, P-50)  
Darbandy S (P-49, P-50)  
Darvish S (P-54)  
Dasgupta R (O-3)  
Datta U (O-3)  
Davodi F (O-10)  
Davoodi D (P-21)  
De Vries JH (O-16)  
Dehghani Firouzabadi R (O-37, P-51)  
Dehghani L (P-27)  
Delpisheh A (Onm-17, P-8, P-68)  
Dickenson DL (I-19, I-20)  
Diedrich K (O-25)  
Djalalinia Sh (Pnm-16)  
Dohle GR (O-16)  
Dubey PK (I-15)  
Dutta Borah BK (P-64)

## **E**

Ebrahimi M (P-52, P-53, P-54)  
Ebrahimi Mamghani M (Pnm-9)  
Eftekhari Yazdi P (P-31)  
Eimani H (P-36)  
Ejtemaemehr S (O-9)  
Elkhayat W (P-46)  
Elton R (O-30)  
Elwagdy S (I-56)  
Engin G (I-2)  
Erfanian Ahmadpoor M (P-55)  
Esmaeili V (O-4)  
Estakhr J (P-69)  
Esteves TC (I-8)  
Exposito A (P-70)  
Ezabadi Z (Onm-5)

## **F**

Faghani M (O-13)  
Fakheri T (I-31)  
Fallahi P (P-45)  
Fallahkarkan M (P-15)  
Fanaei H (P-6)  
Farahmandloo N (P-7)  
Farrokhi A (P-31)  
Fatehmanesh P (O-40)  
Fatemi F (P-39)  
Fatemi Taqatabaie R (P-2)  
Fathi Najafi T (Onm-6)  
Fazeli A (I-30)  
Firoozeh M (P-20)  
Fok KL (O-28)  
Forouhari S (Onm-7)  
Franco Jr. JG (O-15)  
Frith L (I-21, I-22, I-23)

## **G**

Gallego MJ (P-24)  
Garg S (O-22)  
Garret AS (O-2)  
Gentile L (I-51)  
Ghaemi Z (Onm-7)  
Ghafari F (O-11, O-34)  
Ghandi S (P-56)

Ghannadi AR (P-6)  
Ghasemian F (P-23)  
Ghavami F (P-19)  
Ghayour Mobarhan M (P-49, P-50)  
Ghazanfarpour M (Pnm-5)  
Giralt RB (O-32)  
Gispert R (O-32)  
Glasier A (O-30)  
Golestanha A (I-57, I-58)  
Gordon A (P-50)  
Goudarzi M (Pnm-5)  
Gourabi H (Onm-8)  
Guerin JF (O-2)  
Gupta HP (P-34)  
Gupta N (O-12)  
Gupta SC (O-12)  
Gupta VK (O-22)

## **H**

Hafezi Ahmadi MR (P-8)  
Haghani H (P-44)  
Hajibaba Kashani S (Onm-6)  
Hajizadeh RO (Pnm-6)  
Hakim S (P-59)  
Halvaei I (P-25)  
Hamze-Zadeh HR (P-38)  
Hartshorne G (I-24, I-25, O-11)  
Hashemi Z (Pnm-16)  
Hassan Tehrani T (Pnm-10)  
Hassanpoor S (P-33)  
Hassanzadeh M (Pnm-7, Pnm-8)  
Heidari M (Pnm-14)  
Hejazi M (Onm-6)  
Hembram ML (O-3)  
Hooshmandi M (P-19)  
Hossein G (O-36, P-58)  
Hossein GH (P-17)  
Hosseini F (O-34, Pnm-2)  
Hosseini S (P-57)  
Hosseini Salekdeh Gh (I-49)  
Hosseini Z (Onm-4, Pnm-3, Pnm-4)  
Houshmand M (O-47)  
Hsu ChY (P-9)  
Huang LW (P-29)  
Huang MIT (O-31)  
Hübner K (I-51)  
Huseni Balasinor NH (I-50)  
Hwang JL (P-29)

## **J**

Jabbari F (P-23)  
Jacobsson AH (I-9, I-10)  
Jadhav SV (O-5)  
Jafari M (P-5)  
Jahandideh D (P-26)  
Jahanfar S (P-38)  
Jahangiri N (O-34)  
Jahanpour F (P-10)  
Jahanpour NS (P-10)  
Jain S (P-59)  
Jalali S (Pnm-9)  
Jalalsafari M (P-15)

Jaliliyan A (P-68)  
Jannat Alipoor F (O-7)  
Javadi T (P-63, Pnm-22)  
Javdan N (P-69)  
Jimenez C (O-2)  
Johansson L (I-11, I-12)  
Johnstone A (O-30)  
Joniau S (P-9)

## **K**

Kadioglu A (I-2)  
Kakal F (O-45)  
Kalantar SM (O-47)  
Kaltcheva MM (P-24)  
Karimian L (Onm-8)  
Karimy shoar M (P-33)  
Karimzadeh M (O-17)  
Karimzadeh MA (I-40)  
Kashani I (O-9)  
Kavuosi A (P-20)  
Kazemi M (P-27)  
Kazemnejad S (P-17, P-58)  
Kazeroni M (P-6)  
Kedia-Mokashi N (I-50)  
Keshargar S (P-6)  
Keyhaneh M (O-40)  
Khajehjahromi S (O-13, P-11)  
Khalaf Y (I-41, I-42)  
Khalili MA (P-12)  
Khan SA (O-5)  
Khan Y (O-45)  
Khanmohammadi M (P-17, P-58)  
Khazaei M (P-28)  
Khazamipour N (O-40)  
Khodabandeh Jahromi Z (O-41)  
Khole VV (O-5)  
Khoradmehr A (P-12)  
Khorramkhorshid HR (O-10)  
Kiani A (Pnm-14)  
Kiani K (I-31, O-34)  
Ko K (I-51)  
Kokab A (O-6)  
Kumar P (O-3)

## **L**

Lafont C (I-59)  
Lai JN (O-31)  
Lai YC (O-42)  
Lankarani N (O-34, Onm-16)  
Latifnejad Roudsari R (O-18, O-19, P-40)  
Laurenco D (O-43)  
Lee CKF (I-38, I-39)  
Lee KF (I-30)  
Lee WF (O-31)  
Lefèvre CM (O-44)  
Levy R (O-2)  
Li SY (O-42)  
Liao CF (O-31)  
Lin L (O-43)  
Lin YH (P-29)  
Lindemans J (O-16)  
Lornage J (O-2)

Lu LM (O-31)  
Lyashchenko OI (O-33)

## **M**

Macklon NS (O-16)  
Madani T (I-43, O-34)  
Maertens A (O-2)  
Mahdavi A (O-27, P-45)  
Maheshwari A (P-4)  
Mahmoodi F (Pnm-10)  
Mahmoodi L (Pnm-6)  
Malekafzali H (Pnm-16)  
Maleki H (P-38)  
Maleki Porgoo A (Pnm-11)  
Malhotra S (O-22)  
Malik A (P-59)  
Manca R (P-71)  
Mansoori Torshizi M (P-55)  
Mansouri P (P-60)  
Marzban N (P-69)  
Mashayekhy M (O-26)  
Massaro FC (O-15)  
Matorras R (P-70)  
Mauri AL (O-15)  
Mazhaeri Y (P-2)  
Mazochiyan H (P-33)  
McElreavey K (O-43)  
Meabe A (P-70)  
Meethal SV (P-24)  
Mehravar S (P-13, Pnm-12)  
Melvin L (O-30)  
Merghati T (Onm-16)  
Meur SK (I-15)  
Meyer HHD (O-46)  
Minaee B (P-65)  
Minoia G (P-71)  
Mirghavamedin NS (Onm-9)  
Misro MM (P-4)  
Modaresi M (P-14)  
Moghaddam Banaem L (O-20, P-41)  
Mohammad Ghasemi F (O-9, O-13, P-11, P-15, P-23)  
Mohammad K (O-24)  
Mohammadi B (O-20, P-41)  
Mohammadi Kh (Pnm-13, Pnm-14)  
Mohammaditabar Sh (Pnm-13, Pnm-14)  
Mohammed N (O-23, O-45)  
Mohebbi Z (P-60)  
Mohseni H (P-20)  
Mohseni M (P-5)  
Moin MR (P-12)  
Moini A (I-44)  
Moini M (Onm-11)  
Mokaram P (P-49)  
Mollaahmadi F (Onm-10)  
Mollahosseini F (Onm-15)  
Moradi M (Onm-15)  
Morovati H (P-2)  
Moser M (I-13, I-14)  
Moshfegh Z (P-61)  
Moshfeghy Z (P-47)  
Motamednia L (Onm-4, Pnm-3, Pnm-4)

Mousavi S (Pnm-15)  
Movahedin M (O-1, O-38, O-39, P-3)  
Mowla SJ (O-38, O-39)  
Mozaffari M (P-42)  
Muradov Z (I-2)  
Murphy SE (I-27)  
Mutinati M (P-71)

## **N**

Naamavar MR (P-26)  
Nada A (P-46)  
Najeebullah M (O-21, Onm-12)  
Nandan D (P-4)  
Nasiri E (P-11)  
Nasr Esfahani MH (O-14)  
Nazia Sh (O-21, Onm-12)  
Neisani Samani L (Pnm-2)  
Nejad Chehrizi V (O-20, P-41)  
Nematy M (P-49, P-50)  
Nicholas KR (O-44)  
Nicoletti AP (O-15)  
Nikraves A (P-69)  
Noorafshan A (P-19)  
Noruzinia M (O-40)  
Nwaogu IC (P-30)

## **O**

Okolie IP (P-30)  
Oliveira JBA (O-15)  
Omidi A (O-19)  
Ong He Q (O-28)  
Oskouie SFH (P-38)  
Owji AA (P-49)  
Oyen R (P-9)

## **P**

Pandey A (O-12)  
Panjeshahin MR (P-26)  
Pathak ShG (I-50)  
Payman A (O-8, P-43)  
Pelengaris S (O-11)  
Persdotter-Eberg K (Onm-13)  
Petersern CG (O-15)  
Peykari N (Pnm-16)  
Peyman H (Onm-17, P-68, P-8)  
Pfeiffer MJ (I-8)  
Popov A (I-45, I-46)  
Porayette P (P-24)  
Poshtvar F (Pnm-17)  
Poureisa M (I-60)  
Pourkeramati F (O-10)  
Pozzetto B (O-2)  
Prakash BS (P-64)  
Prasad JK (P-34)  
Prasad P (O-48)  
Prasad S (P-34)  
Pujol P (O-40)

## **Q**

Qureshi R (O-45)

## **R**

Rahbar T (O-22)  
Rahmani N (Pnm-18, Pnm-19)  
Rahmati M (O-36)

Rahnama P (Pnm-13)  
Rahnavardi M (P-5)  
Rajabpour Niknam M (P-31)  
Rambod M (P-60)  
Ramezani M (P-12)  
Ramezani Tehrani F (Pnm-16)  
Ramon O (P-70)  
Ranjbar S (P-15)  
Ranpura SA (O-5)  
Rasekh Jahromi A (O-35)  
Rashidi Z (P-28)  
Razazan M (P-61)  
Rizvi M (P-59)  
Rizzo A (P-71)  
Rodrigues BA (P-32)  
Rodrigues JL (P-32)  
Rodriguez P (P-32)  
Roseau G (I-59)  
Roshandel S (O-8, P-43)  
Roshangar L (P-33, P-35)  
Roskams T (P-9)  
Rouzier R (I-59)  
Rowlands DK (O-28)

## **S**

Sabaghiyan E (Onm-6)  
Sabour D (I-51)  
Sabzaligol M (Onm-14)  
Sadeghi M (I-31, Onm-11)  
Sadeghi MR (O-10, P-38)  
Sadeghi Tabar A (O-10)  
Sadighi Gilani MA (P-20)  
Sadri S (P-28)  
Saeidi Nejat Sh (Pnm-21)  
Safarian M (P-49, P-50)  
Safdar M (P-16, P-62, Pnm-20)  
Safdari H (Pnm-21)  
Safdarian L (O-27, P-45)  
Sahraei H (P-27)  
Sahranavard P (P-58)  
Sahranavardfard P (P-17)  
Sajadian AS (Onm-15)  
Saki G (P-18)  
Saki M (P-63, P-63, Pnm-22)  
Saki MA (Pnm-22)  
Salehnia M (P-21)  
Salehpour S (I-47)  
Salehy M (P-27)  
Salman Yazdi R (O-4, O-7)  
Salsabili N (O-17)  
Samani RO (Onm-16, P-42)  
Sanli O (I-2)  
Saoudin H (O-2)  
Saraf Razavi M (P-55)  
Sareban MT (P-60)  
Sarkar M (O-46, P-64)  
Sarmast Shooshtary F (P-65)  
Schams D (O-46)  
Schilffarth S (O-46)  
Schöler HR (I-51)  
Sciorsci RL

Scott G (O-30)  
Sedighi E (P-41)  
Sefiabad Shapouri M (P-22)  
Sehgal N (P-4)  
Seifmanesh HR (Onm-17, P-68, P-8)  
Sekhavat L (O-37, P-51)  
Seow KM (P-29)  
Seyed Fatemi N (Pnm-2)  
Seyedhassani SM (O-47)  
Shahhoseini M (P-31)  
Shahporian L (Onm-4, Pnm-3, Pnm-4)  
Shahriari H (I-28)  
Shahverdi AH (O-4, P-36)  
Shaik J (O-23)  
Shaik N (O-23)  
Shariati M (P-14, P-19)  
Sharifi A (P-44)  
Sharifi E (P-19)  
Sharma GT (I-15)  
Sharma N (O-48)  
Sharma U (O-48)  
Sharp JA (O-44)  
Shayan P (P-1)  
Shetty A (O-6)  
Sheung Chan PK (O-28)  
Shie MC (O-31)  
Shiekh L (O-45)  
Shirvani M (Pnm-17)  
Shishehgar F (O-8, P-43)  
Shokri S (O-9)  
Shukla I (P-59)  
Sills ES (I-26, I-27)  
Silva AEF (P-32)  
Singh B (P-34)  
Singh M (O-48)  
Singh MM (O-22)  
Singh SK (O-48)  
Singhal S (P-34)  
Smith PA (O-18, P-40)  
Soheili F (P-68)  
Sojoodi Moghaddam MH (P-56)  
Soleimani Rad J (P-33, P-35)  
Soleimannejad M (Onm-17)  
Soltan Ghorae H (O-10)  
Soltan Nejad M (P-42)  
Somani M (O-45)  
Spedicato M (P-71)  
Steeegers EAP (O-16)  
Steeegers-Theunissen RPM (O-16)  
Stehling M (I-51)  
Suryawanshi AR (O-5)  
Svoboda P (I-52)  
Sze Ho L (O-28)  
**T**  
Taavoni S (P-44)  
Tabibnejad N (P-56)  
Taganova O (P-66)  
Tahmaseb Pour A (I-61)  
Tardy JC (O-2)  
Tavalaee M (O-14)

Tavana S (P-36)  
Tehraninejad ESh (I-48)  
Tekieh E (P-27)  
Thomassin-Naggara I (I-59)  
Toousi M (Onm-18)  
Torabizadeh A (P-67)  
Torné MM (O-32)  
Totonchi M (P-31)  
Tousi M (Pnm-23)  
Toussy JA (O-29)  
Tripathy R (O-22)  
Trisolini C (P-71)  
Tsang LL (O-28)  
Tzeng CR (P-29)  
**U**  
Ulugberdyeva AA (P-66)  
Upadhyay R (I-1)  
**V**  
Vahidi S (O-24, P-12)  
van der Horst G (I-16, I-17)  
van der Spek PJ (O-16)  
Van Poppel H (P-9)  
Vaziri MH (P-20)  
Viville S (I-53, I-54)  
Vosough Taqi Dizaj A (P-20)  
Vujkovic M (O-16)  
**W**  
Walters E (O-11)  
Wang F (P-37)  
Wang WC (O-42)  
Wu HF (P-37)  
Wylie K (O-6)  
**Y**  
Yadegari Z (Onm-6)  
Yaghoubi M (Onm-17, P-68, P-8)  
Yang J (P-37)  
Yang JJ (O-42)  
Yasemi M (P-8)  
Yavangi M (P-5)  
Yavari Z (Pnm-8)  
Young EH (O-28)  
Young H (O-30)  
**Z**  
Zafar W (O-45)  
Zafari Zangeneh F (P-65)  
Zarabi M. (Onm-19)  
Zeraati H (P-39)  
Zhao B (P-49, P-50)

**Aims and Scope:** The "*International Journal of Fertility & 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 the member of Committee on Publication Ethics (COPE).**

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\* Edelman CL, Mandle CL. Health promotion throughout the life span. ST Louis: Mosby; 1998; 145-63.

\* Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press ;1995;465-78.

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**Abstract book:**

\* Amini rad O. The Antioxidant Effect of Pomegranate Juice on Sperm Parameters and Fertility Potential in Mice. *Yakhteh*.2008; 10 Suppl 1: 38.

**Conferences:**

Hamden P, Joffe JK, Jones WG, editors. Germ cell tumors V. Proceedings of the 5th Germ cell tumors conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.

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