

## Key Lectures

### K-1

#### **Towards an OHSS free clinic - GnRHa trigger state of the ART**

**Humaidan P.**

*The Fertility Clinic, Odense University Hospital, Denmark.*

**Email:** peter.humaidan@ouh.regionsyddanmark.dk

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

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

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

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

### K-2

#### **Can we improve implantation by cancelation of fresh embryo transfer?**

**Al-Hasani S.**

*Reproductive Medicine Unit, University of Schleswig-Holstein at Luebeck, Ratzburger Alle 160, 23538 Luebeck, Germany.*

**Email:** sf\_alhasani@hotmail.com

Single embryo transfer is becoming increasingly popular in IVF/ICSI. More IVF/ICSI cycles therefore include freezing of high quality embryos, and the cumulative effect of such cycles becomes more important. To improve the results obtained using frozen-

thawed embryos, the predictive value of embryo and patient characteristics such as ovarian reserve, hormone levels and age play an important role in both cases whether the women treated with oestradiol/progesterone or undergo natural cycle transfer. Although, embryo quality indicators revealed sometime morphologically and numerically inferior embryo cohorts after cryopreservation, the clinical pregnancy rate is higher in cycles using thawed embryos compared with fresh. Moreover, subsequent logistic regression analysis controlled for differences in embryo quality and revealed significantly greater probability of clinical pregnancy with thawed embryos when compared with fresh, suggesting a negative effect of ovarian stimulation on endometrial receptivity. The aim of this study is to discuss an idea of cancellation of a fresh embryo transfer and put on an alternative method which is the frozen thawed embryo.

### K-3

#### **Quality of life in patients with endometriosis**

**Alborzi S.**

*Department of Obstetrics and Gynecology, Gynecologic Endoscopy Ward, Shiraz University of Medical Sciences, Shiraz, Iran.*

**Email:** alborzis@sums.ac.ir

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

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

Medical treatments are not indicated for patients with endometriosis and infertility but should be considered for those with pain and as an adjuvant after surgical treatment. In those patients with infertility laparoscopic treatment of endometriosis or controlled ovarian

hyperstimulation with intrauterine insemination (COH-IUI) and assisted reproductive technology (ART) are the best modalities. ART is the method of choice for those with severe distortion of tubo-ovarian anatomy. Because hormonal suppressive treatment does not cure endometriosis recurrence or persistence of endometriosis can be expected in nearly all patients after the cessation of medical treatment, and this is positively correlated with the severity of endometriosis. The main goal of laparoscopic treatment of patients with pain is to resect all endometriotic lesions as much as possible. It is the most difficult pelvic operation and should perform by an expert laparoscopist. When endometriosis causes mechanical distortion of the pelvis surgery should be performed to achieve reconstruction of normal pelvic anatomy. Surgical management of minimal and mild endometriosis appears to offer a small, but significant, benefit with regard to fertility outcome. Sometimes patients should be operated by a team of expert gynecologic laparoscopist and urologist or colorectal surgeon especially in these with bowel and ureter involvement.

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

#### K-4

##### Fresh versus frozen embryo transfer in ART

**Aflatoonian A.**

*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** abbas.aflatoonian@yahoo.com

After introducing vitrification technique, we began to use this method for embryo freezing in our centers. Regarding the satisfying results, two studies were performed at Yazd Research and Clinical Center for Infertility and Madar Hospital. Two papers were published of these studies (1, 2).

A meta-analysis based on electronic literature search in MEDLINE, EMBASE, and the Cochrane library was carried out at 2011 (3). The authors included randomized clinical trials comparing outcomes of IVF cycles between fresh and frozen embryo transfer. After the screening of titles and abstracts, leaving seven articles including our articles listed above (1, 2), considered to be eligible for meta-analysis. After the second selection, one of our articles was excluded (2) because it was not RCT and the other (1) was considered as three RCTs included quantitative synthesis. The result of mentioned systematic review confirmed our findings that IVF outcomes may be

improved by transferring frozen embryo compared with fresh embryos. There are recent studies about the effect of frozen embryo transfer on reducing the risk of ectopic pregnancy. Due to the increasing of elective single embryo transfer, it seems that the use of frozen embryos will increase in the future.

In conclusion, the perspective of ART is toward using frozen embryos rather than fresh embryos.

1. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. *J Assist Reprod Genet* 2010; 27: 357-63.
2. Aflatoonian A, Mansoori Moghaddam F, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART cycles. *J Assist Reprod Genet* 2010; 27: 695-700.
3. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil Steril* 2013; 99: 156-162.

#### K-5

##### Individualized controlled ovarian stimulation (iCOS)

**Humaidan P.**

*The Fertility Clinic, Odense University Hospital, Denmark.*

**Email:** peter.humaidan@ouh.regionssyddanmark.dk

**Introduction:** With the recent development of recombinant gonadotropins (FSH and LH), it has become possible to further adjust the stimulation protocol according to the expected needs of the patient. In this respect, the possible beneficial role of exogenous LH activity supplementation for stimulated ART cycles has received increasing attention. According to the two-cell, two- gonadotropin theory (Fevold, 1941), both FSH and LH are required for normal folliculogenesis in humans. LH stimulates the production of androgens in the theca cells, which in turn are aromatised to estradiol by the granulosa cells under the action of FSH. However, at a follicle size of 8-10 mm in normogonadotropic women, the granulosa cell also acquires LH receptors in addition to the FSH receptors, already present. Once LH receptors are expressed in the granulosa cell, LH is able to regulate both steroidogenesis and growth of the follicle; thus, from this moment on FSH function can to a large extent be replaced by LH activity.

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

that two subgroups of normogonadotropic patients: patients >35 years of age (Marrs *et al* 2004, Humaidan *et al* 2004, Bosch *et al* 2008; Matorras *et al* 2009) and patients with an initial sub-optimal response to FSH only preparations (Barrenatxea *et al* 2000; De Placido *et al* 2004; Ferraretti *et al* 2004; Ruvoilo *et al* 2007) seem to benefit from modifications of the stimulation protocol in terms of exogenous LH activity supplementation. Possible biological reasons for a beneficial effect of LH activity supplementation in these sub-groups will be discussed as well as molecular, structural and functional differences between LH and hCG. Finally, the importance- or not of late follicular progesterone rise during COS will be debated.

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

#### K-6

##### Personalized reproductive medicine

Nayernia K<sup>1,2</sup>.

1. Department of Human Genetics and Stem Cell Sciences, Institute for Personalized Medicine (PMed Institute), Dusseldorf, Germany.
2. GENECELL Advanced Molecular and Cellular Technologies, Montreal, Canada.

Email: prof.nayernia@gmail.com

In 2003, the human genome was sequenced completely, and it was indicated that human genome is consisting of 33.000 genes. One of the early hopes of the genomic project was to pinpoint specific genes that caused common diseases. Scientists, physicians and the pharmaceutical industry are actively developing ways to customize medical treatments to suit our unique genetic signatures. This resulted in development of the new field of medicine which termed as personalized medicine.

Personalized medicine is a young but rapidly advancing field of healthcare that is informed by each person's unique clinical, genetic, genomic, and environmental information. Because these factors are different for every person, the nature of diseases- including their onset, their course, and how they might respond to drugs or other interventions- is as individual as the people who have them.

Personalized medicine is about making the treatment as individualized as the disease. It involves identifying genetic, genomic, and clinical information that allows accurate predictions to be made about a person's susceptibility of developing disease, the course of disease, and its response to treatment.

Development of new technologies such as gene Chips and microarray analysis in reproductive medicine allow

the clinicians to determine the molecular profiling of reproductive cells, sperm and oocytes, and embryonic cells, which termed as personalized reproductive medicine. Personalized reproductive medicine support selection of viable embryo(s) and help to maximize the chance of pregnancy by screening the endometrial receptivity. In addition, this new field of reproductive medicine helps us to estimate the overall viability of the cohort of embryos in order to economize the IVF treatment options.

Considering the therapeutic aspects of personalized reproductive medicine, stem cells, in particular umbilical cord blood stem cells are applying to cure embryonic diseases in uterus by using of intrauterine stem cell transplantation which is a promising approach for treatment of human diseases in prenatal stages.

#### K-7

##### Surgical management of endometrium in infertile women

Mehdizadeh A.

Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.

Email: amehdizadehkashi@yahoo.com

Endometrioma is best defined as an ovarian pseudocyst arising from growth of ectopic endometrial tissue, which progressively invaginates the ovarian cortex. It is commonly seen in women of reproductive age who may complain of pelvic pain, dyspareunia and/or subfertility; some others may be asymptomatic. The pathogenesis of infertility in women with endometriosis has not been clearly understood except in cases of distorted pelvic anatomy. Even in women undergoing IVF, pregnancy rates were found to be lower in patients with endometriosis compared with those with tubal factor infertility IVF is the mainstay of treatment for endometriosis-related subfertility. Some gynaecologists would offer surgical removal of endometrioma prior to commencing ovarian stimulation for IVF. The effects of surgical removal of endometrioma on ovarian reserve and ovarian response to gonadotrophin stimulation have been the focus of much studies.

#### K-8

##### Psychological aspects and neurobiology of infertility

Yassini Ardekani SM.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: smya1960@gmail.com

There is a report of more infertility in woman with history of depressive symptoms and lower pregnancy rate in depressed woman in IVF cycles. Beside, psychological interventions may lead to increase pregnancy rate and many references suggest psychological intervention as a program in conjugation

with IVF. In other point of view, treatment failure is not infrequent in IVF centers and it is a stressful event for women that can precipitate serious psychological reactions because it is ranked second as the most severe stress after death of a family member. Critical factor is the number of treatment failure experience per se rather than time spent in treatment. Recent models about stress and infertility contemplate interactions between hypothalamic–pituitary–adrenal (HPA) axis, noradrenergic and adrenergic system, hormonal and neurobiological systems such as the hypothalamic–pituitary–gonadal (HPG) axis or the sympathetic–adrenal–medullar system.

Stress acts through different mechanisms, not only by inhibiting the HPA axis but also by altering the concentration of fertility hormones (FSH, GnRH and LH) as well as other substances such as cortisol, opioids and melatonin. It alters the follicular levels of glucocorticoid hormones and of 11 $\beta$ -HSD. It also affects semen quality. Stress reduction is a non-invasive, less expensive and ethically acceptable way of improving fertility.

#### K-9

#### Developmental sex disorders and male infertility

Zuffardi O<sup>1</sup>, Dehghani MR<sup>1,2</sup>, Vetro A<sup>1</sup>.

1. Department of Molecular Medicine, University of Pavia, Pavia, Italy.
2. Research and Clinical Center of Infertility, Reproductive and Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: zuffardi@unipv.it

The molecular causes of disorders of sex development (DSD) in humans are only partially understood. In particular, only 20% of DSD in subjects with XY karyotype is now specifically bindable to a given mutation/genomic alteration. On the contrary, a specific molecular cause is identifiable in 80% of DSD subjects with XX karyotypes. In fact, in the latter ones, the most frequent causative event is a translocation that carries the distal region of Yp, including the SRY gene, on the tip of Xp. A portion of the so-called XX males is however SRY negative and in a minor portion of them array-CGH analysis showed a microduplication in a desert region of 17q24.3, about 500 kb far from the 5' of the SOX9 gene. Individuals with this duplication may present either with ambiguous external genitalia or with infertility due to azoospermia.

SOX9 gene is activated in the bipotential gonad by the protein encoded by SRY which operates for a short period of time in collaboration to the protein encoded by SF1. The activation of SOX9 in the XY background is self-maintaining thanks to a network between the SOX9 protein and other factors. Mutations of SOX9 and some deletions in the desert region at its 5' are associated with

campomelic dwarfism with XY sex reversal in 75% of patients. Similarly, both transgenic XX mice and the rare XX subjects with the gene's duplication present with sex reversal. The association between genotype and phenotype both in subjects with duplications of various portions of the desert region at the 5' of the gene and with reciprocal translocations involving the same region, has led us to hypothesize that some individuals with XX DSD and the absence of SRY have heterozygous mutations of a silencer element located in the most distal part of the desert region at the 5' of the gene. These mutations should prevent the silencing of SOX9 in the XX background causing its ectopic expression and a partial differentiation of the gonad into testis. The same mutation would not have any phenotypic effects in the XY background and could be inherited from fathers with normal gonadal differentiation.

#### K-10

#### Laparoscopic myomectomy, new technique for myometrial closure

Asefjah H.

Parsian Hospital, Tehran, Iran.

Email: hossein.asefjah@gmail.com

Uterine leiomyomata (UL), also known as fibroids, are benign tumors of the uterus and the leading cause of hysterectomy in the United States, accounting for \$1.2 billion in hospital expenditures annually. Most women develop myomas during their lifetimes; however, 80% are asymptomatic. When symptoms are determined to be caused by myomas, a number of management options exist that include “watchful waiting” medical therapy, surgery, or more recently uterine artery embolization and focused ultrasound. Uterine myoma is a common gynecologic disorder occurring in 20-50% of women of late reproductive age and preservation of fertility is the primary concern. The first lesson physicians must learn is that if the patient is asymptomatic, no treatment is necessary. The presence of an abdominal mass is not an indication for hysterectomy or myomectomy unless it is of significant concern to the patient.

Symptoms vary in severity and include pelvic pain, abnormal menstrual bleeding, and pregnancy complications. The etiology of UL is poorly understood. Increasing incidence of diagnosed UL during reproductive years and decreased incidence with menopause suggest the role of sex steroid hormones. Recently, laparoscopic myomectomy has been advocated because of its small operative wound, short hospital stay, quick recovery, and outcome comparable to traditional laparotomy. Myomectomy, either abdominal or laparoscopic, is an approach particularly suited for those women who wish future fertility. It seems clear that, in well trained and experienced hands, well-selected patients can have myomectomy performed

under laparoscopic direction. Very large myomas are not as suitable for the laparoscopic approach, but laparoscopic myomectomy up to 20 cm has been reported in literature, which solely depends on surgeons ability.

There is no universally accepted criteria regarding number and size of myoma to be removed laparoscopically but as our techniques, especially suturing techniques and instruments for laparoscopy advance, our ability to do more complicated cases of laparoscopic myomectomy increase as well. Before laparoscopic myomectomy uterine mapping is mandatory, because the surgeon does not have sense of palpation during procedure, in order to have successful laparoscopic myomectomy the surgeon should answer the following questions before surgery;

- How many myomas are there?
- Where are the exact location of myomas?
- What is the distance of myoma from cavity?
- Is uterine cavity distorted?
- Are we able to perform operation?

Laparoscopic myomectomy is a challenging procedure and the most challenging part of this procedure is suturing. The goal of suturing is to restore myometrial integrity, prevent hematoma formation, prevention of defect and dehescence in myometrium and adhesion prevention. If any one of these goals are not met during procedure the future pregnancy would be in danger. Skill of surgeon is the most important factor for successful operation. In video clip the new technique for myometrial closure will be displayed.

## K-11

### Prenatal diagnosis of skeletal dysplasias

**Antsaklis A.**

*Ian Donald School, Greek Branch, Athens, Greece.*

**Email:** arisants@otenet.gr

The word *Dysplasia* originates from the ancient Greek words *Dys* (anomalous) and *Plasia* (formation). Skeletal dysplasias' (SD) is a heterogeneous group of congenital anomalies characterized by abnormalities in the development of the bone and cartilage tissues. These diseases may present either in the form of isolated findings or a phenotypic manifestation of a chromosomal aberration or a genetic disorder. Prenatal diagnosis is mainly on the ultrasonographic appearance, which is usually achieved during the second trimester of pregnancy. Two dimensional ultrasonography may detect the majority of SD, however difficulties in the diagnosis as well the differential diagnosis are frequently arising. In such cases, further evaluation is needed by the use of additional imaging modalities or by invasive procedures, in order to detect an underlying chromosomal abnormality or a single gene disorder. Accurate diagnosis is crucial in order to establish successful genetic counseling, as well as appropriate case management. This approach includes the use of three-dimensional ultrasonography and three-

dimensional computed tomography; whereas fetal magnetic resonance imaging is less important. These new imaging modalities have an important role in the prenatal multidisciplinary approach of the diagnosis of SD. Despite the indisputable progress that has been achieved during the last few years, in some cases the antenatal detection of SD delays and is feasible only at the late second or even third trimester. Thus, important ethical and medical issues arise in the antenatal management and counseling of these pregnancies, particularly in the case of lethal SD.

## K-12

### Epidemiology and etiology of infertility in Iran: A systematic review and meta-analysis

**Parsanezhad ME<sup>1</sup>, Namavar Jahromi B<sup>2</sup>, Najaf Zare N<sup>3</sup>, Keramati P<sup>4</sup>, Khalili A<sup>5</sup>.**

1. *Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
2. *Infertility Research Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Department of Biostatistics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
4. *Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*

**Email:** parsanezhad@msn.com; meparsanezhad@rogers.com; parsame@rogers.com

**Introduction:** Epidemiological study of infertility might let the related policy makers to make accurate decisions regarding the potential users' requirements for infertility workup or management. To find the incidence and etiologic factors for infertility in Iranian population.

**Materials and Methods:** An internet-based search through PubMed, Google Scholar and Iran Medex was performed restricted to the publications in the recent 25 years (1987-2012) in English or Persian. This project included all of the studies that were designed by random cluster sampling with face to face interviews at home from the Iranian population. Life time infertility was defined as inability to conceive after 12 months of unprotected intercourse. For analyzing the causes of infertility we included the published articles that were designed by Iranian infertility clinics and evaluated the causes of infertility by appropriate diagnostic techniques. Independent data extraction was performed by two observers and meta-analysis was done. Random effects meta-analyses, a forest plot, publication bias and sensitivity analyses were performed.

**Results:** Twelve studies that were designed to evaluate the prevalence rate of infertility were identified and meta-analysis was performed to integrate the findings of the separate studies. The average rate of infertility was; 10.9% (95% CI 7.4-14.4), primary infertility; 10.6% (95% CI 5.3-16.0), secondary infertility; 2.7% (95% CI 1.9-3.5) and current infertility; 3.3% (95% CI 2.7-3.8).

Causes of infertility were picked up from seven qualified studies. Male factor was; 34.0% (95%CI 26.9-42.0), female factor; 43.5% (95% CI 35.5- 51.7), both male and female factors; 17.1% (95% CI 11.4-21.9) and unexplained cause; 8.1% (95% CI 5.6-11.5), respectively.

**Conclusion:** Prevalence rate of life time infertility was 10.9%. The most common cause was female factor.

## K-13

### New update on PCOS (2012)

**Salehpour S.**

IVF Center, Infertility and Reproductive Health Research Center, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

**Email:** saghar.salehpour@yahoo.com; z\_sadeghzadeh2001@yahoo.com

PCOS is a common hormone disorders affecting ovaries, adrenals, pancreas, liver, muscle, blood vasculature and fat.

Women with PCOS are at risk of type 2 diabetes, high cholesterol and high blood pressure. Obesity worsen the condition. The degree of obesity vary by ethnicity.

- There are 3 sources for diagnoses of PCOS:
  - Rotherdam
  - NIH
  - AE Society
- Limitations of AE criteria's:
  - Blood measurement
  - Differences between laboratories
  - Ethnical difference in clinical androgen excess
- limitations of ovulatory dysfunction:
  - Normal ovulation is not understood.
  - Ovulatory dys function is difficult to measure
- Limitations of PCOS morphology.
  - Technique dependent
  - Lack of standard through the cycle
- Etiologies:
  - Genetics
  - Epigenetic
  - Psychologic factors
  - Life style
- Prevention:
  - Life style
  - CC
  - Letrozole
  - Treatment of sleep apnea

## K-14

### DNA repair signaling pathway genes are overexpressed in complex aneuploid preimplantation embryos

**Gourabi H<sup>1</sup>, Bazrgar M<sup>1,2</sup>, Eftekhari Yazdi P<sup>1</sup>, Vazirinasab H<sup>1</sup>, Fakhri M<sup>1</sup>, Hassani F<sup>1</sup>, Valojerdi MR<sup>1</sup>.**

1. Royan Institute for Reproductive Biomedicine (ACECR), Tehran, Iran.

2. Sciences and Culture University, Tehran, Iran.

**Email:** gourabi@royaninstitute.org

**Introduction:** Gene expression of DNA damage pathways is common in preimplantation stage. This study aims to explore the altered expression of DNA damage signaling pathways including apoptosis, cell cycle, and DNA repair functional gene groups in embryos with aneuploidies.

**Materials and Methods:** The study was performed on day-4 surplus embryos from PGD candidates who gave their informed consent. The embryos were pooled into two groups. Group 1 included good quality embryos that had a single aneuploidy, only one chromosome according to the FISH based PGD of day-3, a normal rate of cell division, and grades A-B (excellent to good). Group 2 included embryos with the following characteristics: more than one aneuploid chromosome in day-3 PGD, an abnormal rate of cell division, and grades C-D (fair to poor). We analyzed gene expression of DNA damage signaling pathways by commercial real-time PCR-based array, which included 84 genes of interest after specific preamplification of cDNA by a primer mix, included all genes of array. In each group, 3 biological replicates and 2 technical replicates from each biological replicate were studied. Five housekeeping genes were considered in order to select the best housekeeping gene for data normalization. Expression fold differences between groups were calculated using the 2- $\Delta\Delta C_t$  method. The p-values were calculated based on a Student's t-test.  $p < 0.05$  was considered significant.

**Results:** One hundred three out of 437 surplus embryos were included in this study, 46 in the Group 1 and 57 in the Group 2. We chose ribosomal protein L13a (RPL13A) as the best housekeeping gene for data normalization from the five general house-keeping genes of the array. Overexpression was significant for 5 out of 84 studied genes ( $p < 0.05$ ) in the Group 2 included MutS homolog 3 (MSH3); X-ray cross-complementing group 1 (XRCC1); RAD50 homolog (RAD50); ligase 1, DNA, ATP-dependent (LIG1); and cyclin-dependent kinase 7 (CDK7). These overexpressed genes in the Group 2 belong to DNA repair functional gene group. We observed marginal expression differences in five other genes ( $p < 0.1$ ); however expression of genes involved in cell cycle and apoptosis signaling pathways were not statistically different between groups.

**Conclusion:** Among the studied genes of DNA damage signaling pathways, we have shown that MSH3, XRCC1, RAD50, LIG1, and CDK7 (which are involved in DNA repair) upregulated in complex aneuploid human preimplantation embryos. It seems that in comparison with the cell cycle control and apoptosis, DNA repair is more activated in complex aneuploid embryos.

### K-15

#### Benefits of the prenatal diagnosis and preimplantation genetic diagnosis in recurrent pregnancy loss

Ghasemi N.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: n479g@yahoo.co.uk

Recurrent pregnancy losses (RPL) could cause psychological and physical disorders. The etiology of 50% of the cases is not found, which causes the fear of the results of the next pregnancy. Prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) could help couples with idiopathic RPL. The suggesting invasive PND in carrier couples is poorly followed, especially in carrier couples with maternal age over 35 years. However, non-invasive PND could help them medically also psychologically. The encouraging carrier couples for doing invasive PND procedures should be the topic for further research to give better clinical care and instructive decision making. In-vitro fertilization clinics could employ PGD in women with recurrent pregnancy loss. PGD should be successful in treating couples with balanced chromosomal abnormality. It could reduce pregnancy failure in women more than 35 years in age with a normal karyotype. However, this procedure is not usually beneficial in younger patients. In general, women with RPL produced more abnormal embryos than control groups.

### K-16

#### Genetic/Genomic studies of consanguinity in developing countries

Romeo G.

University of Bologna, Medical School and European School of Genetic Medicine, Italy.

Email: romeo@eurogene.org

The application of the most recent DNA sequencing technology to consanguinity studies show that the causative mutation for any rare autosomal recessive disorder can be identified by whole exome sequencing if only two affected sibs born of first cousins can be studied (Pippucci *et al*: Hum Hered 2011; 72: 45-53). But consanguinity is also a powerful for establishing the prevalence of rare disorders in inbred population groups. Classical studies of consanguinity have taken advantage of the relationship between the gene frequency for a rare autosomal recessive disorder (q) and the proportion of offspring of consanguineous couples who are affected with the same disorder. We recently developed a new approach for estimating q using mutation analysis of affected offspring of consanguineous couples based on the possibility that the child born of consanguineous parents carries the same mutation in double copy (true homozygosity) or alternatively carries two different mutations in the same

gene (compound heterozygosity) inherited through two different ancestors.

The proportion of compound heterozygotes among children affected with a given autosomal recessive disorder, born of consanguineous parents, can be taken therefore as an indirect indicator of the frequency of the same disorder in the general population. Data from the offspring of consanguineous marriages affected with different autosomal recessive disorders collected by different molecular diagnostic laboratories in Mediterranean countries where the frequencies of consanguineous marriages is high, show the validity of this approach which we called the HI (Homozygosity Index) method (Gialluisi *et al*: Ann Hum Genet 2012;76: 159-67). In particular we tested the HI method on different samples of patients affected with two autosomal recessive disorders, namely Familial Mediterranean Fever (FMF) and Phenylketonuria (PKU), born either to first cousins or to unrelated parents. More recently the HI method has been used to verify the high prevalence of Wilson disease (WD) in Sardinia where essentially four different mutations in the ATP7B gene (13q14.3, MIM 606882) are associated with alterations of copper metabolism resulting in pathological progressive copper accumulation in liver and other tissues. The worldwide prevalence (P) of WD is about 30/million, while in Sardinia it has been estimated to be in the order of 1/10000, one of the highest worldwide (Loudianos *et al*: Hum Mutat 1999; 14: 294-303).

All these estimates have been inferred through classical clinical approaches, and as such they are likely to suffer from an underdiagnosis bias. Indeed, a recent molecular neonatal screening in Sardinia reported a WD gene frequency of 1.92% with a resulting prevalence of 1/2707 live births (Zappu *et al*: Pediatr Gastroenterol Nutr 2008; 47:334-338). The HI method (see above), applied to a sample of 178 carefully characterized patients collected by the pediatricians of the Ospedale Regionale per le Microcitemie and University of Cagliari, confirms the results reported by Zappu *et al* raising the interesting question of whether the high prevalence of WD in Sardinia is due to genetic drift or selection.

In conclusion the widespread use of the molecular genetic analysis of causative mutations to confirm clinical diagnoses has considerably improved the possibility of using the consanguinity approach based on the HI method which has become reliable, accurate and inexpensive. The results of this type of studies can be used to establish priorities for screening and intervention policies, as in the case of WD in Sardinia. This is not a trivial result for communities where autosomal recessive "rare" disorders can be not so rare and have a strong social impact.

### K-17

#### The medicine within the genome: the great option of next generation sequencing

Zuffardi O<sup>1</sup>, Deghani MR<sup>1,2</sup>, Vetro A<sup>1</sup>.

1. Department of Molecular Medicine, University of Pavia, Pavia, Italy.

2. *Research and Clinical Center of Infertility, Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** zuffardi@unipv.it

Molecular diagnosis of genetic diseases is important especially in a pediatric setting in order to 1. Establish the recurrence risk in following pregnancies, 2. Stop the diagnostic odyssey that is frequently restless for undiagnosed diseases, and 3. Provide, at least in some cases, specific therapeutic treatments to achieve improved outcomes or more accurate prognosis. In theory, the molecular diagnosis should offer the pharmacologists a great advantage to study patients homogeneous in respect to the causative gene rather than to the clinical phenotype even if deeply detailed by biochemical and instrumental investigations (magnetic resonance, electrocardiogram, electroencephalogram, and etc). In turn this should allow the investigation of the specific protein or protein pathway alteration, permitting clinical trials for new drugs in categories of homogeneous patients.

Presently, the great enthusiasm coming from the efficiency of high-throughput sequencing (next generation sequencing, NGS) in elucidating the cause of heritable disorders has "infected" many fields of medicine. Although this strategy doesn't magically make diagnoses but typically provides a handful of possibilities that may require further functional studies, NGS is indeed a great option providing answers to those patients whose mysterious conditions have long eluded diagnosis. Applications of this technique to non invasive prenatal diagnosis promise to radically change any traditional approach.

## **K-18** **Fertility preservation**

**Eftekhar M.**

*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** maryameftekhar@yahoo.com

Due to improvements in cancer treatment, cure rates of adult and childhood cancers improved considerably over the past three decades. But, long term consequences of cancer therapy and effect on quality of life are now being known. One of the most important effects of cytotoxic chemotherapy is gonadal failure. This presentation summarizes available options and Specific strategies for fertility preservation in women with malignancies. The best approach depends on the type of cancer, the type of treatment (e.g., radiation and/or chemotherapy), time existing till onset of treatment, age of patient and whether the patient married. The suggestion for fertility preservation include: fertility sparing surgery, Ovarian transposition, in vitro fertilization (IVF) with embryo cryopreservation, oocyte cryopreservation for later IVF, ovarian tissue cryopreservation The tissue may be autotransplanted to

pelvic, when the patient improved, to attempt spontaneous conception or subcutaneously and then follicles is aspirated for IVF. Otherwise, it can be xenografted to mice to induce follicle maturation in preparation for aspiration for IVF. Other treatment options for fertility preservation include medication to reduce chemotherapy-induced oocyte damage.

## **K-19** **Diabetes and the impairment of male reproductive function**

**Talebi AR.**

*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Email:** Prof\_talebi@hotmail.com

Diabetes mellitus (DM), is a chronic disorder of the carbohydrate, lipid and protein metabolism, characterized by insulin disorders, hyperglycemia and glycosuria. Type 1 and type 2 diabetes are on the increase throughout the world, with the latter increasingly being described as a "modern disease" caused by lifestyle, diet and obesity. DM will involve additional men prior to and during their reproductive years. It is suggested that diabetic men are less fertile than non-diabetics. About 90% of diabetic male patients have disturbances in sexual function including decrease in libido, impotence or erectile dysfunction (50-75% of cases) and infertility. The results of recent studies have shown that in addition to decrease in semen volume, sperm normal morphology and motility, these patients showed higher rates of sperm DNA damage in comparison with healthy fertile men. On the other hand, the deletions in the sperm mitochondrial DNA had been shown to be associated with DM. A number of studies have suggested that high level of mtDNA deletions in sperm cells is linked to lower fertility in men. Deletions and fragmentation of DNA result in loss of genetic material and may cause infertility as the sperm is not able to deliver its full complement of genetic codes in fusion with the egg to create a viable embryo. So, it would be important to understand the mechanism by which these sperm damage occurs in diabetics.

Increased production of mitochondrial reactive oxygen species (ROS) following hyperglycemia is recognized as a major cause of the clinical complications associated with DM and obesity. It should be noted that spermatozoa are particularly susceptible to oxidative stress because their plasma membranes contain large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of scavenging enzymes as antioxidant system. However, ROS has two targets including plasma membrane and DNA in the spermatozoa and other cells. Since, the process of apoptosis relates to ROS production, the apoptosis signalling, measured by disrupted transmembrane mitochondrial potential and activated caspase 3, is



significantly increased in sperm from males with diabetes type-I and type-II. Also, alterations in mitochondrial DNA seen in diabetes are responsible for the adverse changes observed in motility of spermatozoa.

In conclusion, there are several subcellular factors to explain infertility or subfertility in diabetic males. These factors are; disrupted transmembrane mitochondrial potential, fragmentation and deletion in mtDNA, oxidative stress, high levels of sperm DNA damage and apoptosis.

## K-20

### Cryopreservation of ovarian tissue

**Salehnia M.**

*Department of Anatomy, Tarbiat Modares University, Tehran, Iran.*

**Email:** mogdeh@dr.com, salehnm@modares.ac.ir

Cryopreservation of human ovarian tissue using slow cooling/rapid thawing methods has been used successfully and until now 28 babies were born by this technique. Another simple and low cost alternative technique for preservation of ovarian tissue is vitrification. Vitrification of ovarian tissue has some problems in comparison with single cells, because the ovarian tissue has heterogeneous cellular component and different cell types that have different diffusion rates of cryoprotectants. Moreover the ovarian tissue has fibrous stroma especially in large mammalian species such as bovine and human and the penetration of cryoprotectant within this fibrous tissue seems to be difficult. Thus cryopreservation of ovarian tissue needs more attention to oocytes, follicular cells and stroma. More studies recently have been focused to improve the quality of vitrification of human ovarian tissue by changing on the carrier systems and increased in cooling rate however the information on the effects of vitrification methods on the fine structure and incidence of apoptosis within human ovarian tissues remain limited and some of them are controversial therefore further investigation is required.

## K-21

### Culture media for human preimplantation embryos

**Mastenbroek S.**

*Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherland.*

**Email:** S.Mastenbroek@amc.uva.nl

Subfertility is of major clinical, social and economical concern. The most frequently used interventions are in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Despite their frequent use, over 600.000 cycles were reported in 2002, the two largest data collections only report a delivery rate per started cycle of 18.4 and 25.2% respectively. For a successful

pregnancy to occur, good quality preimplantation embryos are essential. Several studies have suggested that culture media have an impact on the quality of embryos generated in IVF/ICSI cycles, thereby influencing implantation and pregnancy rates.

By performing a systematic review and meta-analysis we indeed showed the potential importance of culture media for treatment outcome. However, conventional meta-analysis was not possible for any of the outcomes in this review as nearly all trials that were included compared different culture media. Therefore, it is yet unknown what culture medium leads to the best success rates in IVF/ICSI. Another search of the literature that we performed focusing on specific components of culture media and their effect on IVF/ICSI treatment outcomes showed similar inconclusive results.

Recent studies have indicated that the type of culture media used to culture human preimplantation embryos in IVF/ICSI can affect the birth weight of newborns. In addition, animal data suggested that culture media affect gene expression and the imprinting status of bovine and mouse embryos. This suggests that the in vitro culture of human embryos might have prolonged effects on the health of offspring, similar to the effect of in utero undernutrition on disease susceptibility in adulthood which was shown in our center before when examining prenatal exposure to the Dutch famine in relation to disease later in life.

To study to what extent the transcriptome of human embryos is affected by in vitro culture conditions, we randomized human preimplantation embryos to two culture-media (G5-medium or HTF-medium) and to two oxygen concentrations (5% or 20%), with stratification for maternal age. Next to these variables, developmental stage after culture was also taken into account in the analysis of the gene expression profile of these embryos, as an effect of developmental stage on the transcriptome profile of human embryos had been demonstrated before. Developmental stage and maternal age appeared to be the two main factors leading to differences in the transcriptome profile of human preimplantation embryos. In vitro conditions (culture medium and oxygen concentration) had a more subtle effect on the transcription profile of embryos. Interactions between the factors were found, indicating that culture conditions might have a different effect depending on the developmental stage or the maternal age of the embryos.

Given the potential importance of culture media for treatment efficacy and the health of children being born, rigorously designed RCTs are needed for currently available, as well as newly introduced culture media.

## K-22

### Role of sperm transcripts in oocyte activation, fertilization and development

**Nasr-Esfahani MH<sup>1,2</sup>, Tavalaei M<sup>1</sup>.**

1. *Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.*

2. *Isfahan Fertility and Infertility Center, Isfahan, Iran.*

**Email:** mh.nasr-esfahani@royaninstitute.org

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

### K-23

#### Mitochondrial DNA mutations and cancer

**Romeo G.**

*University of Bologna, Medical School and European School of Genetic Medicine, Italy.*

**Email:** romeo@eurogene.org

In the last decade studies on mitochondria and cancer have revealed a high frequency of mitochondrial DNA (mtDNA) mutations in tumors leading to revisit Warburg's hypothesis. Certain tumor subtypes are characterized by abnormal accumulation of nonfunctional mitochondria in their cytoplasm and tumors which develop such a phenotype are called oncocytoomas. They usually arise in tissues of epithelial origin such as thyroid, kidney or pituitary gland, but have also been reported in breast, endometrial, lung and colon cancers.

In order to progress towards malignancy, any solid tumor must go through hypoxic adaptation, a process tightly connected with metabolic reactions and directed by a transcription factor called Hypoxia Inducible Factor 1  $\alpha$  (HIF1 $\alpha$ ). Oncocytoomas however, because of their inability to perform mitochondrial respiration, are not able to adapt to hypoxia and therefore generally maintain a low-proliferative, non-invasive state. Genetic changes underlying the benign nature of oncocytoomas are so the called disruptive mtDNA mutations which when present in homoplasmy, i.e. in all mtDNA copies of a tumor cell, abolish mitochondrial respiration and prevent HIF1 $\alpha$  assembly and therefore the subsequent progression to malignancy. However this anti-tumorigenic effect of disruptive mtDNA mutations is

not observed when they are present in heteroplasmy in the cancer cell, thus giving rise to the concept of the double-faceted effect (oncojanus) of such mutations.

The identification of oncojanus mtDNA mutations not only can improve the management of oncocytoma patients and enable appropriate recognition of these often under-diagnosed tumors, but also sets the ground for the development of novel complex I targeted therapeutic strategies against cancer in general.

### K-24

#### GIFT and ZIFT

**Aleyasin A.**

*Infertility Center, Shariati Hospital, Tehran, Iran.*

**Email:** ivfshariati69@gmail.com

IVF is one of the most important techniques for treatment of infertile couples. IVF-ET and gamete intra fallopian transfer (GIFT) is applied for treatment of tubal factor and unexplained infertility since 1970. GIFT was developed as a method is transfer oocyte and sperm via laparoscopy to tube. In this method fertilization before tubal transfer is unknown. Devroey described the first successful zygote intrafallopian transfer (ZIFT) in 1986. In this method fertilization is occurred before embryo transfer. ZIFT is applied in patients who have failed GIFT but in all couples who have at least one patent fallopian tube.

In recent years modified techniques for tubal transfer of embryo is reported according to developmental stage of the embryos being transferred.

This techniques include:

1. PROST
2. TPET
3. TEST
4. TET

We do rapid ICSI-ZIFT at oocyte retrieval.

Advantages of ZIFT:

1. Embryo development occurs in the natural and physiological environment of the fallopian tube.
2. Better synchronization between embryonic and endometrial development.
3. Prevention of microtrauma to the endometrium by uterine transfer catheters.

Disadvantages:

1. Risks associated with general anesthesia laparoscopy and longer hospital stay.
2. Increased cost
3. Increased risk of ectopic pregnancy

Recently ZIFT technique is applied for treatment of infertile couples with:

1. Repeated IVF failures
2. Difficult trans cervical embryo transfer
3. Advanced maternal age

## K-25

### Antioxidant and male infertility

Moein MR.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: moein1339@yahoo.com

Male infertility can have several causes. The most common is idiopathic oligoasthenoteratozoospermia (OAT). Despite extensive research, a successful treatment for OAT has not yet been developed. Seminal oxidative stress (OS) results from an imbalance between reactive oxygen species (ROS) production and ROS scavenging by seminal antioxidants. Seminal OS is believed to be one of the main factors in the pathogenesis of sperm dysfunction and sperm DNA damage in male infertility. Indeed, it is estimated that 25% of infertile men possess high levels of semen ROS, whereas fertile men do not have high levels of semen ROS. Excessive production and/or reduced clearance lead to oxidative stress within sperm, resulting in DNA damage, reduced motility and defective membrane integrity. Antioxidants may help maintain the balance between ROS production and clearance and could thus improve sperm quality. Observational studies have also found a lower frequency of sperm aneuploidy in men with a higher dietary intake of antioxidants than in those with a lower intake. Infertile men have higher levels of semen ROS than do fertile men. High levels of semen ROS can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. This observation has led clinicians to treat infertile men with antioxidant supplements.

To date, most clinical studies suggest that dietary antioxidant supplements are beneficial in terms of improving sperm function and DNA integrity. Antioxidant supplementation could be useful in increasing the scavenging capacity of seminal plasma, but would not treat the underlying condition that causes the reduced fertility. While there is a good body of literature on the effect of oral antioxidants on sperm parameters (including sperm DNA integrity), no study has established the optimal dose, duration of treatment or subpopulation of infertile patients who might benefit most from antioxidant therapy (isolated asthenozoospermia, oligoasthenoteratozoospermia, sperm DNA damage or all). The improvement in sperm parameters resulting from antioxidant therapy may result in a higher pregnancy rate, but this is not consistent and the possibility of negative effects on sperm DNA, capacitation and the acrosome reaction should be carefully evaluated. Furthermore, not all individuals are equally eligible for antioxidant therapy,

and not all are likely to benefit in the same way from the same treatment.

## K-26

### Menopausal women's sexual function: effect of Ginkgo Biloba, a Randomized placebo controlled trial

Taavoni S<sup>1,2</sup>, Amiri M<sup>1</sup>, Seyedfatemi N<sup>1</sup>, Haghani H<sup>1</sup>.

1. Tehran University of Medical Sciences, Tehran, Iran.

2. Research Institute for Islamic and Complementary Medicine (RICM), Tehran University of Medical Sciences, Tehran, Iran.

Email: staavoni14@yahoo.com

**Introduction:** Sexual health affects women's and their spouse's quality of life. There are several studies that show sexual dysfunction is more prevalent among menopausal women and need to prevent and treat it by using effective pharmacological or none pharmacological methods with less side effects. This study was designed to identify effect of Ginkgo Biloba Extract (GBE) on menopausal women's sexual function.

**Materials and Methods:** In this triple blind randomized placebo controlled trial, 80 healthy menopause volunteers' with age 50-60, whom had been admitted in three health care centers of Tehran University of Medical Sciences (TUMS), involved. (Year 2010-2011) Tool of study had two main parts of personal characteristics, and Sabbatsberg Sexual Rating Scale (SSRS), which was used for subjective evaluation of the sexual function before and after intervention. The participants received GBE 120-240 mg or placebo daily for 30 days. (40 participants in each group) All ethical points were considered and registered in Iranian Registry of Clinical Trials (IRCT).

**Results:** Equality of two groups according to participants' age, spouses' age, age in Last menstruation, number of children and number of coitus in month checked. The most domains of sexual function included sexual desire ( $p<0.05$ ), sexual pleasure ( $p<0.01$ ), orgasm ( $p<0.05$ ), importance of sex ( $p<0.01$ ) during last month and importance of sex in comparison to previous years ( $p<0.007$ ) were significantly improved in the GBE group. The mean score of total sexual function in the GBE group had significant difference with placebo group ( $p<0.05$ ).

**Conclusion:** Findings of this study support positive effect of Ginkgo Biloba on menopausal women's sexual function.

## Award Winners

### A-1

#### Assessing the efficacy of aspiration and ethanol injection in recurrent endometrioma before IVF cycle

Rahmani E<sup>1</sup>, Rahsepar M<sup>2</sup>, Aflatoonian A<sup>2</sup>.

1. Department of Obstetrics and Gynecology, Bushehr University of Medical Sciences, Bushehr, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: rahmani@bpums.ac.ir

**Introduction:** Endometriosis is a common hormone-dependent gynecologic disease with a high recurrence. Laparotomy or laparoscopy is the standard surgery for the large endometrioma. Also, sclerotherapy is basically used to treat different diseases one of which is endometrioma. The study was designed to assess the value of transvaginal ultrasound-guided ethanol sclerotherapy in patients.

**Materials and Methods:** In a randomized clinical trial, an interventional group of 20 patients underwent transvaginal ethanol sclerotherapy for recurrent ovarian endometrioma. The patients were followed up first after one and two weeks and then after one, two, and three months. If the patients had no endometrioma, they were treated with IVF (standard long protocol). A control group of 20 patients with endometrioma were enrolled for an IVF protocol. They had no treatment by ethanol sclerotherapy. IVF parameters, pregnancy rates, and implantation rates were compared in both groups.

**Results:** The demographic data showed no difference between the two groups. The initial mean endometria size was 41.45±15.9 cm, the recurrence rate after 6 months was 4 (20%), FSH before and after sclerotherapy was 6.97±2.25 (IU/L) and 6.78±1.88 (IU/L) p=0.343. The clinical pregnancy rate was 6 (33.3%) vs. 3 (15%), p=0.616. The fertilization rate emerged 63.06% vs. 60.38%, p=0.57. The implantation rate turned out 12.9% vs. 7.5%, p=0.52. None of these results were significant. However, the data pointed to a better trend toward the ethanol sclerotherapy group.

**Conclusion:** Ethanol sclerotherapy is an effective strategy for the treatment of recurrent endometrioma especially before IVF.

**Key words:** Recurrent endometrioma, Ethanol sclerotherapy, In vitro fertilization, Vaginal ultrasonography, Guided aspiration.

### A-2

#### In vitro generation of male germ cells from ram marrow-derived mesenchymal stem cells

Ghasemzadeh-Hasankolaei M<sup>1,2</sup>, Batavani R<sup>2</sup>, Sedighi-Gilani MA<sup>3</sup>, Baghaban MB<sup>1</sup>.

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary

Medicine, Urmia University, Urmia, Iran.

3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

Email: ghasemzadeh78@gmail.com

**Introduction:** In recent years some researchers have suggested that bone marrow-derived mesenchymal stem cells (BM-MSCs) have the capability of differentiation into the germ cells (GCs). The aim of this research was to evaluate the possibility of in vitro production of GCs from BM-MSCs using some BM-MSCs were isolated from ram bone marrow and then they were divided into some groups.

**Materials and Methods:** These groups were three different concentrations of retinoic acid (RA in 1, 5 and 10 µM), TGFb1 (10 ng/ml), BMP4 (100 ng/ml), BMP8b (100 ng/ml) and zinc sulphate (ZnSO<sub>4</sub> 0.14 µM) that treated for 21 days for induction of GC differentiation. At the end of the treatment period, cells were evaluated for expression of GCs-specific characteristics. Evaluations was consisted of assessment of the cells' morphological changes, GC-specific gene expression VASA, PIWIL2, OCT4, ITG b1, DAZL by RT-PCR and quantitative RT-PCR, and PGP 9.5 by immunocytochemistry and alkaline phosphatase activity.

**Results:** It was found that all three concentrations of RA can induce differentiation into GCs in BM-MSCs and 10 µM was the most effective concentration. TGFb1 could well produce some cells (6.2%) with GCs characteristics in the culture of BM-MSCs. BMP4 and BMP8b were almost weak inducers and after 21 days treatment, the percentage of cells with GCs characteristics were 0.37% and 0.51%, respectively. In ZnSO<sub>4</sub> group, evaluations showed that zinc could not induce GCs differentiation in BM-MSCs but it has a regulatory effect on the expression of some GC-specific genes.

**Conclusion:** Totally, according to the results, it can be concluded that in vitro production of male GCs is possible but more studies should be performed to achieve the optimum procedure for production of functional GCs.

**Key words:** Marrow-derived mesenchymal stem cells, Differentiation, Male germ cells, In vitro.

### A-3

#### Hormonal regulation of TLRs in human fallopian tube cells

Zandieh Z<sup>1</sup>, Amjadi FS<sup>1</sup>, Ashrafi M<sup>2</sup>, Aflatoonian A<sup>3</sup>, Fazeli A<sup>4</sup>, Aflatoonian R<sup>2</sup>.

1. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4. Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, United Kingdom.

Email: R.Aflatoonian@gmail.com

**Introduction:** Toll Like Receptors are the main family of pattern recognition receptors. They recognize pathogen-associated molecular patterns and constitute a major part of the innate immune system. Previously it was reported that expression of these receptors are altered in the female reproductive tract during different stages of menstrual cycle. Here we used a fallopian tube epithelial cell line (OE-E6/E7) for investigating the role of sex hormones in modulating TLRs expression in fallopian tube.

**Materials and Methods:** Initially TLRs expression in OE-E6/E7 cells was compared to that of fallopian tube tissue to validate the use of these cells as a model for investigation of TLR expression in the fallopian tube. To investigate the effect of sex hormones on the expression of TLRs 1-6 in fallopian tube epithelial cell line (OE-E6/E7), initially TLRs 1-6 expression in OE-E6/E7 cells was compared to that of fallopian tube tissue with RT-PCR and immunostaining. Thereafter OE-E6/E7 cells were cultured with different concentrations of estrogen (0.1, 1, 10, 100 nM) and progesterone (1, 10, 100, 1000 nM) and combination of them, control (C) (without any additional treatment of sex hormone), menstruation (M) (1nM progesterone and 0.1 nM estradiol), pre-ovulation (P) (6.5 nM progesterone and 1.5 nM estradiol) and window of implantation (W) (35 nM progesterone and 1 nM estradiol), in 5% CO<sub>2</sub> atmosphere in 75 ml flasks 24 hours in the absence of phenol red and serum. Quantitative polymerase chain reaction was performed to reveal any changes in TLRs gene expression as a result of hormonal treatment.

**Results:** TLR1-6 genes were expressed in human fallopian tube tissue and TLR 1-6 genes and proteins were expressed in OE-E6/E7 cell line. Although estrogen and progesterone had no significant effect on TLRs expression alone, combination of them altered the expression of TLRs in OE-E6/E7 cell line.

**Conclusion:** In conclusion, the present investigation firmly points to involvement of combination of estrogen and progesterone in modulation of TLRs gene expression in human fallopian tube cells. Further experiments should reveal the regulatory mechanism and signaling pathway behind this effect of sex hormones in modulating innate immunity in the human female reproductive tract.

**Key words:** Estrogen, Fallopian tube cells, Innate Immunity, Progesterone, Toll-Like receptors.

#### A-4

**Comparing the efficiency of different culture systems on proliferation and purification of the spermatogonial stem cells from obstructive azoospermic patients**

Zahiri M<sup>1</sup>, Movahedin M<sup>1</sup>, Mowla SJ<sup>2</sup>, Noruzinia M<sup>3</sup>.

1. Department Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

2. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

3. Department Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Email: mmovahedin@yahoo.com

**Introduction:** Spermatogenesis is a highly organized process that is tightly regulated. Spermatogonial stem cells (SSCs) are responsible for Spermatogenesis. Studying about the biology of SSCs provides better understanding about male infertility, germ cell cancer and male contraception.

**Materials and Methods:** As the mechanisms that involved in human spermatogenesis are complex and unknown, we evaluate different cultural Colonization of isolated human SSCs were studied in various groups during 2 weeks culture. Equal number of cell population in each group was sorted with MACS for GFR- $\alpha$ 1 antibody and the other part was not sorted. Both groups were cultured for further one week. Gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, Oct-4) and specific germ cell) Integrin  $\alpha$ 6, Integrin  $\beta$ 1, PLZF) genes in each stages were evaluated by MSP and quantitative PCR. To revealing functionality, spermatogonial cells from the selected group were transplanted to azoospermia mouse model. Colonization of isolated human SSCs were studied in various groups during 2 weeks culture. Equal number of cell population in each group was sorted with MACS for GFR- $\alpha$ 1 antibody and the other part were not sorted. Both groups were cultured for further one week. Gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, Oct-4) and specific germ cell) Integrin  $\alpha$  6, Integrin  $\beta$  1, PLZF) genes in each stages were evaluated by MSP and quantitative PCR. To revealing functionality, spermatogonial cells from the selected group were transplanted to azoospermia mouse model.

**Results:** The results showed that the number and diameter of colonies in testicular cell suspension was significantly higher than others ( $p \leq 0.05$ ). The expression of germ specific genes in testicular cell suspension and after purification was significantly increased ( $p \leq 0.05$ ). Nanog and C-Myc expression level were significantly decreased in this group ( $p \leq 0.05$ ). There was no significant difference about the expression of Oct-4 among testicular cell suspension and other groups ( $p > 0.05$ ).

**Conclusion:** Gene specific methylation pattern of examined genes didn't show any changes during culture period. Our data from transplantation indicated the homing of the donor derived cells and the presence of human functional sperm. In conclusion our results confirmed that culture of testicular cell suspension and selection of spermatogonial cells could be effective ways for purification and enrichment of the functional human spermatogonial cells.

**Key words:** Spermatogonial stem cells, Enrichment, Colony formation, Co-cultivation, Transplantation.

## A-5

### The effect of immature oocytes quantity on ICSI outcome

Halvaei I<sup>1</sup>, Khalili MA<sup>1</sup>, Razi MH<sup>1</sup>, Nottola SA<sup>2</sup>.

1. Department of Embryology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Anatomy, Histology, Forensic Medicine and Orthopedics, La Sapienza University, Rome, Italy.

Email: ihalvaei@gmail.com

**Introduction:** Several studies reported the effect of the number of oocytes retrieved on ART outcome. However, studies about the effect(s) of the number of immature oocytes retrieved on ART outcome are scarce. The goal was to evaluate the role of the number of retrieved immature oocytes on mature oocyte counts and.

**Materials and Methods:** 101 ICSI cycles were included in this prospective evaluation. morphology, and also the rates of fertilization, embryo development, pregnancy and delivery rate in ICSI cycles. Patients were divided into 2 groups of A ( $\leq 2$  immature oocytes) and B ( $> 2$  immature oocytes). In subanalysis, the impacts of the number of GV and MI oocytes were assessed on the rates of fertilization and embryo sub-analyzed development. Also, correlations between the numbers of immature and mature oocytes, as well as maternal age between two groups were analyzed. Assessments of oocyte morphology, fertilization, embryo quality and development were done accordingly.

**Results:** There was no correlation between the immature oocytes pregnancy and delivery rate quantity with the number of mature ones. There were insignificant differences for embryo development between two groups, but fertilization rate was higher in group A ( $p=0.03$ ). In sub-analysis, insignificant differences were observed between two groups of  $\leq$  and  $>2$  GV and MI oocytes for rates of fertilization and embryo development. Also, the rates of clinical pregnancy and delivery were insignificant between groups. The rate of morphologically abnormal oocytes had no significant difference between two groups, except for wide perivitelline space (PVS) which was higher in group A ( $p\leq 0.03$ ). There was no significant difference for maternal age between two groups.

**Conclusion:** In cases with few retrieved immature oocytes, rates of fertilization and incidence of wide PVS may increase, although immature oocytes may not have any negative impacts on early embryo development or the rates on number of mature oocytes.

**Key words:** Immature oocytes, Oocyte morphology, Fertilization rate, Embryo quality, ICSI.

## A-6

### Does $\alpha$ -Tocopherol and L-carnitine increase CatSper expression in aging mouse testis?

Mohammadi Sh<sup>1</sup>, Jalali M<sup>1</sup>, Nikravesh MR<sup>1</sup>, Fazel AR<sup>1</sup>, Ebrahim Zadeh AR<sup>1</sup>, Sankian M<sup>2</sup>, Gholamin M<sup>2</sup>.

1. Department of Anatomy and Cell Biology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

2. Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Email: Jalali@mums.ac.ir

**Introduction:** CatSper1-4 is a gene family that expressed uniquely in the testis and related to male fertility and sperm motility. Studies show an increase in both sperm motility and gene expression in mammalian cells following vitamin E+ L- carnitine treatment. The aim of this study was the effects of vitamin E plus L-carnitine on CatSper gene expression

**Materials and Methods:** Forty-eight aging male mice 12 months old and forty-eight adult male mice 2 months were used in this study (exp.1: aging male mice treated with vitamin E+L- carnitine, exp.2: adult male mice treated with vitamin E+L- carnitine). Experimental groups of male mice were received intra-peritoneally vitamin E+L- carnitine (106 mg/kg  $\alpha$ -tocopherol acetate plus 50 mg/kg L-carnitine) for 5 weeks. Mice scarified by cervical dislocation at days 21, 28 and 35 after injection. Testes and epididymis were collected from each group. Real- Time PCR was performed for both CatSper and L-actin genes. Sperm parameters consist of sperm count, motility, morphology and viability rate was evaluated based on WHO guideline for human sperm examination and eosin staining. Immunohistochemistry used for tracing of the CatSper protein in sperm. Firth method was used for grade staining (from light to dark brown). The grading was done by two separate observers. Data were analyzed using SPSS soft ware and ANOVA.

**Results:** The expression of CatSper genes was determined using Real-Time PCR. The results showed that there is a significant increase in gene expression for experimental groups when compared to the control ones. The finding of sperm analysis demonstrated that sperm parameters improve in aging and adult male mice following of vitamin E+ L-carnitine treatment when compared to the control group. Immunohistochemistry staining showed the location of CatSper protein in sperm tail.

**Conclusion:** combination of vitamin E +carnitine in aging cases can improve sperm quality. Also, this treatment can increase CatSper gene expression which is one of the responsible genes in motility of sperm.

**Key words:** Gene expression, CatSper, Tocopherol, Aging, carnitine.

## A-7

### Zona pellucida birefringence and meiotic spindle visualization of human oocytes are not influenced by in-vitro maturation technology

Omidi M, Khalili MA, Ashourzadeh S, Rahimipour M.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd Iran.

Email: omidi.marjan@yahoo.com

**Introduction:** In-vitro maturation (IVM) is a promising treatment option for certain infertile women. Nowadays,

with the aid of the Polscope, it has become possible to evaluate zona pellucida (ZP) and spindle as parameters of oocyte quality. The goal was to investigate the relationship between the presence of the meiotic spindle and ZP birefringence with morphology of the in-vivo and in-vitro matured human oocytes.

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

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

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

**Key words:** In vitro maturation, ZP birefringence, Spindle, Oocyte morphology, Polscope.

## A-8

### Therapeutic effects of Spermatogonial stem cells transplantation on ischemic testicle following unilateral testicular torsion in mice

Azizollahi S<sup>1</sup>, Aflatoonian R<sup>1,2</sup>, Sedigi-Gilani MA<sup>3,4</sup>, Tajik N<sup>5,6</sup>, Asghari-Jafarabadi M<sup>7</sup>, Koruji M<sup>1,6</sup>.

1. Department of Anatomical Sciences, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Urology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

5. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
6. Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.
7. Department of Statistics and Epidemiology, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran.

Email: skoruji@tums.ac.ir

**Introduction:** Testicular ischemia is the main consequence of testicular torsion, from both clinical and experimental points of view. Obtaining, storage and transplantation of SSCs may be a new idea in treatment of infertility in testicular ischemia following testicular torsion.

**Materials and Methods:** In the present study, the beneficial effect of SSCs transplantation in ischemic testis also assessment of stem cell niche Animals were randomly divided into four groups (n=24): 1) control, 2) sham, 3) torsion model, 4) SSCs-transplanted. The transplanted group had 2 h testicular torsion and was then treated by SSCs transplantation. Isolated SSCs from neonatal mice (age: 2-6 day) were cultured and identified by flowcytometry (ckit-, integrin  $\beta 6+$ ) and RT-PCR for spermatogonial marker (Oct4, GFR $\alpha$ -1, PLZF, Vasa, ITGA6 and ITGB1), then transplanted in to ischemia reperfusion testicle, two weeks post-surgery. The mice were sacrificed by cervical dislocation 8 weeks after SSCs transplantation. The SSCs transplanted testes and epididymides were removed for evaluation of sperm analysis, weight, histopathological evaluation and pre-post meiotic gene expression.

**Results:** The findings indicated that all evaluated parameters (epididymal sperm parameters, Johnsen Score, expression of Plzf, Gfr $\alpha$ -1, Scp-1, Tekt-1 genes and histopathological evaluation) significantly decreased following testicular ischemia reperfusion (group 3) in comparison with control group (p $\leq$ 0.05), whereas those parameters significantly increased in SSCs transplanted animals in comparison with torsion model (group 3). Although listed parameters value obtained after SSCs transplantation were still significantly lower than sham and control groups.

**Conclusion:** SSCs transplantation could up-regulate the expression of pre and post meiotic genes in testicular ischemia and therefore results in improvement of testicular function and structure in testicular torsion.

**Key words:** SSCs, Pre and Post meiotic genes, Testicular torsion.

## A-9

### The role of VEGF and VEGF receptors in recurrent spontaneous abortion

Amirchaghmaghi E<sup>1,2</sup>, Rezaei A<sup>1</sup>, Moini A<sup>2</sup>, Roghaei MA<sup>3</sup>, Maracy MR<sup>4</sup>, Salman Yazdi R<sup>5</sup>, Aflatoonian R<sup>2</sup>.

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.



3. Department of Gynecology and Obstetrics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Departments of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.
5. Department of Andrology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: r.aflatoonian@gmail.com and Rezaei@mui.ac.ir

**Introduction:** Recurrent spontaneous abortion (RSA), as one of main complications of pregnancy, is usually defined as three or more consecutive pregnancy losses before 20<sup>th</sup> week of gestation. In some cases, in spite of extensive work up, the cause of RSA remains unknown so named unexplained RSA. On the other hand, vascular endothelial growth factor (VEGF) is a potent angiogenic factor. It was shown that VEGF with its receptors (VEGFR1, 2) play important roles in fetal angiogenesis and development. The aim of present study is to investigate VEGF and VEGF Receptors gene expression in endometrium of patients with unexplained RSA in compared to normal fertile women. In addition, serum VEGF concentration was compared between these two groups of women.

**Materials and Methods:** Endometrial and blood samples were obtained between day 19<sup>th</sup> and 24<sup>th</sup> of menstrual cycle (window of implantation) from 10 women with unexplained RSA and 6 fertile women who had at least one successful pregnancy. VEGF and VEGFRs gene expression was studied by RT-PCR and then quantified by real time PCR. Normalization of these genes expression was obtained by using beta-actin as housekeeping gene. Relative VEGF and VEGFRs expression quantities were compared between two groups. Enzyme linked immunosorbent assay (ELISA) was used for serum VEGF assay. The p value less than 0.05 was considered as significant level.

**Results:** VEGF and VEGFRs gene expression was detected in endometrium of women with unexplained RSA and fertile ones. The mean relative expression of VEGF gene was lower in women with RSA in compared to normal fertile women while both VEGF receptors were expressed higher in endometrium of women with RSA. In addition, the serum level of VEGF was significantly higher in RSA women in compared to normal fertile ones.

**Conclusion:** Alteration in expression of VEGF and VEGFRs might have an important role in pathogenesis of unexplained RSA.

**Key words:** Vascular endothelial growth factor, Recurrent spontaneous abortion, Vascular endothelial growth factor.

## A-10

### The effect of vitrification on ultrastructure of human in-vitro matured germinal vesicle oocytes

Shahedi A<sup>1</sup>, Khalili MA<sup>2</sup>, Hosseini A<sup>3</sup>.

1. Department of Anatomy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2. Department of Anatomy, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Cell Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: abas\_shahedi2006@yahoo.com

**Introduction:** Vitrification of oocytes as a novel technology of cryopreservation facilities the long-term storage of oocytes for patient in danger of losing ovarian function. In addition, it is a substitute for embryo conservation avoiding associated ethical concerns. The objective was to describe the possible effects of cryotop vitrification on maturation rate and ultrastructural morphology.

**Materials and Methods:** A total of 301 surplus immature GV oocytes obtained from infertile patients were allocated into two groups: (i) GV oocytes (n=150) matured in-vitro (fIVM); and (ii) GV oocytes (n=151) that were first vitrified, then matured in-vitro (vIVM). Supernumerary fresh in-vivo matured oocytes (n=10) were used as control. The maturation media was Ham's F10 supplemented with FSH+LH and human follicular fluid. After 36h of incubation, the oocytes were investigated for nuclear maturation and ultrastructural changes using transmission electron microscopy (TEM).

**Results:** Oocyte maturation rates were reduced (P<0.001) in vIVM (45.92%) in comparison with fIVM oocytes (75.33%). The rate of degeneration was also significantly higher in vIVM than fIVM group (44.4% vs 6.0%). Large and numerous mitochondria and minute vesicle of smooth endoplasmic reticulum (SER) complexes (MV complexes) were observed in both fIVM and vIVM groups. In addition, TEM revealed a drastic reduction in amount of cortical granules (CGs) at the cortex of vitrified-warmed GV oocytes, as well as appearance of vacuoles and small mitochondria-SER aggregates in the ooplasm.

**Conclusion:** Vitrification procedure is associated with ultrastructural alterations in specific oocyte microdomains, presumably related to the reduced competence of cryopreserved oocytes to maturation. This information emphasizes the need for further works on advancing the cryotechnology of human oocytes.

**Key words:** Cryopreservation, GV oocyte, Vitrification, Ultrastructure, In vitro maturation.

## A-11

### Prolonged incubation of processed human spermatozoa will increase DNA fragmentation

Nabi A, Khalili MA, Halvaei I.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: ali.nabi67@yahoo.com

**Introduction:** One of the causes of failure in ART is sperm DNA fragmentation which may be associated with long period of spermatozoa incubation at 37°C. The objective was the evaluation of sperm DNA fragmentation using the sperm chromatin dispersion (SCD).



**Materials and Methods:** In this prospective study, we analyzed twenty one normozoospermic specimens. Semen analysis was performed according to WHO guidelines. The samples were incubated in 37°C after preparation by direct swim-up. DNA fragmentation were assessed at different time intervals (0, 1, 2 and 3h) using SCD test. In this test, after an acid incubation and subsequent lysis, those sperm cells without DNA fragmentation show big or medium-sized halos of dispersion of DNA loops from the central nuclear core. Otherwise, those spermatozoa containing fragmented DNA either shows a small halo, exhibit no halo with solid staining of the core, or show no halo and irregular or faint stain of the remaining core.

**Results:** The rates of normal morphology and progressive motility after sperm processing were  $72.33 \pm 2.53\%$  and  $90 \pm 1.02\%$ , respectively. The rate of sperm DNA fragmentation was significantly higher after 2h ( $8.81 \pm .93\%$ ,  $p=0.004$ ) and 3h ( $10.76 \pm .89\%$ ,  $p<.0001$ ) of incubation compared to 0h ( $4.38 \pm 0.8\%$ ). Also there was positive correlation between the incubation time and sperm DNA damage ( $p<0.0001$ ).

**Conclusion:** Prolonged incubation of prepared normozoospermic samples at 37°C is associated with higher rates of sperm DNA fragmentation. Therefore, it is recommended to limit the sperm incubation to 2h in ART program.

**Key words:** Sperm DNA fragmentation, SCD test, Normozoospermia, Incubation.

## A-12

### Ethical performance in delivery of sexual and reproductive health services: A Delphi study focused on the right of confidentiality

Farajkhoda T<sup>1</sup>, Latifnejad Roudsari R<sup>2</sup>, Abbasi M<sup>3</sup>.

1. Department of Midwifery, School of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Midwifery, School of Nursing and Midwifery, Patient Safety and Health Quality Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Shahid Beheshti Medical Law and Ethics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: farajkhoda\_t@yahoo.com

**Introduction:** Advancement in technology has brought notable changes in current methods of diagnosis and treatment in Sexual and Reproductive Health (SRH), which itself has created ethical issues including confidentiality protection for the clients and health care professionals to deal with in SRH services. The goal was to develop an ethical guideline regarding confidentiality in SRH services in Iran.

**Materials and Methods:** The study was a sequential exploratory mixed method study, which was carried out in two phases between March 2010 and August 2012. In the first phase of the study through a modified three rounds Delphi study the professional code of ethics was

developed. In the Round 1 Delphi, 45 Iranian academics and clinicians were purposively selected from four universities of medical sciences. Data were collected through sending a questionnaire including open-ended questions by Email and responses were analyzed using conventional content analysis. In the Round 2 Delphi, the draft of code of ethics developed in Round 1, delivered electronically to the participants who had taken part in the first Round. After data collection face and content validity (0.94) were calculated. The results of the Round 3 (Consensus percentage 94.98%) was accepted as professional code of ethics towards confidentiality in SRH services for SRH providers.

**Results:** The panelists' views were organized to five categories related to confidentiality including 1) right to confidentiality 2) access to information 3) secure management of client's data 4) third party interests and 5) legal and illegal disclosure.

**Conclusion:** This study has set up an ethical guideline towards confidentiality in SRH services. It can guide health care providers to what they should do and should not do ethically in practice regarding confidentiality in SRH care services. It may be used as a useful tool by all providers who serve women health in Iran and other countries with similar background in Eastern Mediterranean Region too.

**Key words:** Sexual and reproductive health, Confidentiality, Ethical guideline.

## A-13

### Effect of calcium ionophore on fertilization and pregnancy rate in infertile patients with teratospermia candidate for intracytoplasmic sperm injection

Janati S<sup>1,2</sup>, Eftekhari M<sup>1</sup>, Aflatoonian A<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Dezfoul University of Medical Sciences, Dezfoul, Iran.

Email: Sjanati@gmail.com

**Introduction:** Chemical activation is the most frequently method used for artificial oocyte activation, resultant in high fertilization rates. The aim of this prospective randomized controlled study is evaluating the effect of calcium ionophore on fertilization and pregnancy rate in infertile men suffer from teratospermia.

**Materials and Methods:** Total 38 women with teratospermic partner selected for this randomized clinical trial. Patients divided into two groups using table of random numbers. They candidate for Intracytoplasmic sperm injection (ICSI) undergoing with antagonist protocol at our center for treatment of infertility from April till March 2012. Three hundred and thirteen oocyte metaphase 2 selected for ICSI were used. In control group (n=145 oocyte) as routine ICSI was performed. In intervention group (n=168 oocyte) after oocytes retrieval, immediately ICSI was done and,

they were entered to culture media plus 5 mic/ml calcium ionophore for 5 minutes, and washed at least 5 time by MOPS solution .After 16-18 hours they will be evaluated for fertilization.

**Results:** Thirty- eight ICSI cycles were included in this study. There were no significant differences of demographic characteristics and semen parameters between two groups. There were significant differences in total embryo number and fertilized embryos in cases in compare with control group ( $p=0.04$ ,  $p=0.04$  respectively). The fertilization and cleavage rate was not significant different in the activated oocytes group in compared with control group (84.4, 87.74 vs. 95.33, 89.56% respectively). Implantation rate was higher in study group in compare with control group but was not significant (7.4 vs. 17.64%  $p=0.14$ ). There was no significant differences were observed between them in chemical and clinical pregnancy rate (16.7, 16.7 vs. 47.1, 41.2%  $p=0.07$ ,  $p=0.14$  respectively). There were no significant differences in spontaneous abortion rate ( $p=0.22$ )

**Conclusion:** pregnancy rate is increased in teratospermic couples using calcium ionophore. However, in this study the different was not significant, because of small sample size.

**Key words:** Intracytoplasmic sperm injection, Calcium ionophore, Oocyte activation, Fertilization rate.

#### A-14

#### Evaluation of intrauterine injection of recombinant human chorionic gonadotropin before embryo transfer in improvement of implantation and pregnancy rates in in vitro fertilization/intracytoplasmic sperm injection: a prospective randomized controlled trial

Zarei A, Parsanezhad ME, Younesi M, Alborzi S, Zolghadri J, Samsami A, Amooee S, Aramesh S.

*Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*

**Email:** younesi.dr@gmail.com

**Introduction:** Evidence has accumulated that Interleukin-1  $\alpha$  (IL-1 $\alpha$ ) and IL-1 $\beta$  as well as hCG are secreted by the blastocyst and that these agents can have positive effect on the endometrium and endometrial receptivity. The aim of this study is to evaluate the complementary effects of hCG on endometrial receptivity.

**Materials and Methods:** To evaluate the effectiveness of intrauterine injection of recombinant human chorionic gonadotropin (rhCG) before embryo we evaluated 555 patients who referred for IVF/ICSI at the reproductive medicine center of Mother and Child Hospital. All patients were assigned to ART for the first time. One-hundred eighty two patients fulfilled the study. The study group ( $n=182$ ) received 250  $\mu$ g of rhCG ( $n=85$ ) intrauterine administration 12 minutes

before ET. The control group ( $n=97$ ) underwent ET without rhCG.

**Results:** The IR and PR were statistically significantly higher in the 250  $\mu$ g rhCG group (39.0% and 35.4%, respectively) as compared with the control group (23.7% and 20.6%, respectively).

**Conclusion:** Intrauterine injection of 250  $\mu$ g of rhCG before ET statistically significantly improved the implantation and pregnancy rates in IVF/ICSI.

**Key words:** Intracytoplasmic sperm injection, Implantation rate, Intrauterine rhCG, In vitro fertilization, Pregnancy rate.

#### A-15

#### Effect of diabetes on sperm parameters and chromatin quality in mice

Mangoli E<sup>1</sup>, Talebi AR<sup>2</sup>, Anvari M<sup>1</sup>, Pourentezari M<sup>1</sup>.

1. *Department of Biology and Anatomical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

2. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** prof\_talebi@hotmail.com

**Introduction:** Diabetes mellitus (DM), primary or idiopathic is a chronic disorder of the carbohydrate, lipid and protein metabolism. DM may impact male reproductive function at several levels. It is shown that DM has detrimental effects on sperm parameters in human and experimental animals. The aim of this study was to observe the effects of diabetes on sperm parameters (viability, count, morphology and motility) and evaluation of sperm chromatin quality in mice.

**Materials and Methods:** Totally twenty adult male Syrian mice were divided randomly into 2 groups ( $n=10$ ). The animals of group A were considered as controls while group B mice were diabetic that received a single dose (200 mg/kg) streptozotocin (STZ) intra peritoneally. After 35 days, the cauda epididymis of each diabetic mouse was dissected and placed in culture medium for 30 min. The swim-out spermatozoa were analyzed for count, motility, morphology and viability. The sperm chromatin quality and DNA integrity, was evaluated with Aniline Blue (AB), Toluidine blue (TB), Acridine orange (AO) and Chromomycin A3 (CMA3) staining.

**Results:** In sperm analysis, the diabetic mice had poor parameters in comparison with control animals ( $p=0.000$ ). Regarding sperm chromatin quality, the results of TB and AO tests showed statically significant differences between two groups, but in AB and CMA3 staining, we didn't see any differences between them.

**Conclusion:** The results showed that STZ-induced diabetes mellitus may influence the male fertility potential via affecting sperm parameters and DNA integrity in mice. However, according to our data, the diabetes doesn't have any detrimental effects on histone-protamines replacement during the testicular phase of sperm chromatin packaging.

**Key words:** Sperm chromatin, Diabetes, Mice.

**A-16**

**StuI polymorphism on the androgen receptor gene is associated with recurrent spontaneous abortion**

**Jahaninejad T, Ghasemi N.**

*Department of Medical Genetics, Research and Clinical Centre for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** jahani1987@yahoo.com

**Introduction:** Recurrent spontaneous abortion (RSA) is a reproductive problem that occurs in women in reproductive age. It is defined as two or more repeated pregnancy losses before the 20th week of gestation. The risk of miscarriage is enhanced by a variety of factors including chromosomal abnormalities, uterine abnormalities, hereditary thrombophilia, immunologic factors, infections, environmental factors and endocrinologic disorders such as androgen receptor disorders.

**Materials and Methods:** This is a case-control study to

determine whether G1733A polymorphism of androgen receptor gene is associated with RPL. A total of 85 women with at least two recurrent spontaneous abortion before 20<sup>th</sup> week of gestation composed the study group. Subjects were genotyped by the polymerase chain reaction restriction fragment length polymorphism method.

**Results:** The observed frequencies of GG, GA and AA genotypes of the G1733A polymorphism were 5.89%, 82.35% and 11.76%, respectively, for the patient group and 71.76%, 23.51% and 4.71%, respectively, for the control group. Allele frequencies of the G1733A polymorphism among patients and controls were 0.47 and 0.84, respectively, for the dominant allele (G) (wild type) and 0.53 and 0.16, respectively, for the A allele (mutant type).

**Conclusion:** These results indicated that the androgen receptor G1733A polymorphism is strongly associated with increased risk for RSA.

**Key words:** Recurrent spontaneous abortion, Androgen receptor, Polymorphism, RFLP PCR.

## Oral Presentations

### O-1

#### The role of Toll like receptors in recurrent spontaneous abortion

Amirchaghmaghi E<sup>1,2</sup>, Rezaei A<sup>1</sup>, Moini A<sup>2</sup>, Roghaei MA<sup>3</sup>, Maracy MR<sup>4</sup>, Hafezi M<sup>2</sup>, Aflatoonian R<sup>2</sup>.

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Gynecology and Obstetrics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Departments of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.

Email: r.aflatoonian@gmail.com; Rezaei@mui.ac.ir

**Introduction:** Recurrent spontaneous abortion (RSA) is usually defined as three or more consecutive pregnancy loss before 20<sup>th</sup> week of gestation. Despite of extensive work up in some cases of RSA, the etiology remains unknown which called unexplained RSA. Immunological factors are considered as main reason of unexplained RSA. It was shown that innate immunity plays important roles in normal pregnancy. One of the most critical components of innate immunity is Toll like receptors (TLRs) which include at least 10 functional proteins in human. TLRs activation could induce inflammatory responses. In addition, the expression of TLRs has been shown at different parts of human being especially female reproductive tract and tissues related to pregnancy. The aim of present study was to investigate the gene expression of TLR1-10 in endometrium of women with unexplained RSA in compared to normal fertile women.

**Materials and Methods:** Endometrial samples were obtained between day 19<sup>th</sup> and 24<sup>th</sup> of menstrual cycle (window of implantation). From 10 women with unexplained RSA and 6 normal fertile women who had at least one successful pregnancy. After RNA extraction and cDNA synthesis, reverse transcriptase -PCR (RT-PCR) and quantitative PCR were performed using the prepared cDNA and primers for TLR 1-10. Beta actin was used as housekeeping gene. Relative TLRs expression quantities were compared between two groups.

**Results:** TLRs gene expression was detected in endometrium of patients with unexplained RSA and normal women. The mean relative expression of TLR1-10 gene was significantly higher in endometrium of women with unexplained RSA in compared to fertile ones.

**Conclusion:** Increased expression of TLRs may play role in pathogenesis of unexplained RSA. One of potential explanation may be the ability of TLRs in

inducing inflammatory responses since inappropriate inflammation is detrimental for pregnancy. These findings need to be confirmed in protein level and also by studies with larger sample size.

**Key words:** Innate immunity, Toll like receptors, Recurrent spontaneous abortion, Endometrium.

### O-2

#### Human sperm vitrification vs rapid freezing: effects on sperm parameters, DNA fragmentation and hyaluronan binding assay

Agha-Rahimi A, Khalili MA, Nabi A, Ashourzadeh S.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: Azam\_agherahimi63@yahoo.com

**Introduction:** Human Sperm vitrification is a new method that has been introduced recently. The aim was to compare rapid freezing with vitrification of normozoospermic ejaculates. This study surveyed the effects of these methods on rates of sperm parameters, hyaluronan binding assay (HBA), and DNA fragmentation after thawing. Also was evaluated, the effect of cryoprotectant agent (CPA) on vitrification method in another subgroup.

**Materials and Methods:** The experiment was carried out on 30 ejaculated prepared using swim-up technique. Each suspension was divided into four equal parts: 1. control (fresh); 2. Rapid freezing; 3. Vitrification (two subgroup) a. vitrification method (without use CPA) b. vitrification with permeable CPA. For rapid freezing, cryovial of sperm suspension was hold first above surface of liquid nitrogen. For vitrification, 30 µl sperm suspension was dropped into liquid nitrogen directly.

**Results:** The data showed that the rates of progressive motility and viability was  $86.56 \pm 5.91$  and  $95.80 \pm 3.90$  in fresh samples respectively. These parameters declined significantly ( $p=0$ ) to  $40.03 \pm 12.97$  and  $63.21 \pm 7.65$  in rapid freezing, also  $41.90 \pm 10.27$  and  $64.40 \pm 9.97$  in vitrification method. Normal morphology significantly decreased after freeze in all groups. DNA fragmentation index (DFI) in control was  $11.60 \pm 4.47$ . After cryopreservation, DFI was significantly higher in rapid freeze ( $16.60 \pm 5.59$ ,  $p=0.01$ ). In vitrification group, DFI was higher than control, but this increasing was insignificant. The rates of DFI between the two aforementioned freezing groups was insignificant ( $p=0.19$ ). The HBA test was similar between control and cryopreserved groups. Addition of cryoprotectants agents in vitrification method had neutral effect.

**Conclusion:** In conclusion, vitrification is a good and reliable technique for sperm cryopreservation. It has great potential for application in clinical ART cycles, and does not require the cryoprotectants with possible toxicity.

**Key words:** Human sperm, Rapid freezing, Vitrification, Sperm parameters.

### O-3

#### Human Wharton's Jelly Stem Cells express Nucleostemin, Oct4, Jumonji Histone Demethylases 1a and 2c

Ebrahimi B<sup>1</sup>, Forouzannia SKh<sup>1</sup>, Yaghoobi MM<sup>2</sup>, Dehghani Firoozabadi A<sup>1</sup>, Tabatabaee SA<sup>1</sup>.

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biotechnology, Research Institute for Environmental Sciences, International Center for Sciences, High Technology and Environmental Sciences, Kerman, Iran.

Email: Ebrahimi.b@ssu.ac.ir

**Introduction:** Histone methylation at gene promoters modulates transcriptional activity contribute to stem cell fate decisions and renewal. Jumonji Histone Demethylases (JHDMs) Jmjd1a and Jmjd2c are the primary downstream targets of oct4 in ESCs. Jmjd1a and Jmjd2c have been shown to positively regulate Tc1 and Nanog expression respectively by demethylation of the repressive H3K9Me2 and H3K9Me3 marks respectively. These JHDMs maintain the "stemness" state in ES cells. The aim of present study is to report the expression of two histone demethylases in Human Wharton's Jelly Stem Cells (hWJSCs) in the transcription level.

**Materials and Methods:** The aim of present study is to report the expression of two histone demethylases in Human Wharton's Jelly Stem Cells Briefly Blood vessels were removed from the cords and Wharton's Jelly were cut into small pieces with a scalpel and the fragments were digested with collagenase type II (Invitrogen). The obtained cell solution after washing was cultured and maintained in alpha-MEM supplemented with %15 fetal bovine serum (FBS), 100U/mL penicillin, 100 µg/mL streptomycin, and 25 ng/mL amphotericin B and incubated in 5% CO<sub>2</sub> in a 37°C incubator. Total RNA was extracted using the RNeasy kit (Qiagen) treated with DNase I and first strand cDNA was synthesized using M-MuLV-RT enzyme. PCR was accomplished by primers specific for Nanog, Gnl3, Oct-4, Jmjd1a, Jmjd2c and β-actin using Taq DNA polymerase (Cinnagen). The PCR products were run on 1% agarose gel electrophoresis and visualized after EtBr staining.

**Results:** RT-PCR results showed that the ESC markers, Gnl3, Nanog, Oct4, Jmjd1a and Jmjd2c were actively expressed in hWJSCs. This is the first report of jmjd1a, and jmjd2c expression in hWJSCs.

**Conclusion:** The Pluripotency Network contains key regulators that are involved in control of cell cycle, DNA repair and RNA processing and histone modification. Oct4 activate Jmjd1a and Jmjd2c expression and products of these genes maintain Tc1 and Nanog promoters in a permissive state by demethylation of the repressive marks. Findings demonstrate pluripotency-associated gene expression in

human WJSCs which make these cells a potentially clinical source of stem cells for future cell based therapies and tissue engineering due to their ready availability, high proliferation rates, plasticity and tolerance in allogeneic transplantation.

**Key words:** Wharton's Jelly Stem Cells, Pluripotency, Jmjd1a, Jmjd2c.

### O-4

#### In vitro maturation, fertilization, and embryo development of immature human oocytes

Ashourzadeh S, Khalili MA.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: sareashoorzadeh@yahoo.com

**Introduction:** In vitro maturation (IVM) could be used as an alternative choice for treatment of infertile women with polycystic ovary syndrome and poor responders to ovarian stimulation, also as one of the strategies for fertility preservation. IVM oocytes have the potential to develop from fertilized embryos into a fetus. However, the developmental competence of fertilized embryos from IVM oocytes was still lower compared with those matured in vivo. In the present study, the maturation competency of immature (GV and MI) oocytes and MII oocytes spindle dynamic, fertilization rate and development of embryos were compared.

**Materials and Methods:** Immature human oocytes (GV/MI) were cultured for up to 48 hours in commercial IVM medium (SAGE In Vitro Maturation Media) at 37°C in 5% O<sub>2</sub>, 5% CO<sub>2</sub> and 90% air with high humidity. Maturation was considered when they had the first polar body. Matured oocytes were screened for existing of mitotic spindles with Polar Aide Microscope. Oocytes fertilized by intra cytoplasmic sperm injection (ICSI). After ICSI, normal fertilization was confirmed when two distinct pronuclei were present 16-18 hours later and further cleavage was analyzed up to blastocyst formation.

**Results:** 140 GV oocytes and 68 MI oocytes were obtain from 61 ICSI cycles. The overall maturation rate was 67.6% in GV and 75.1% in MI oocytes and their fertilization rates were 61.8% and 45.5% respectively. Mitotic spindle was existed in 39.5% of matured GV oocytes compared with 28.6% of matured MI oocytes. The cleavage rates were higher in GV (59.3%) than MI oocytes (14.3%) however; only 7.5% of embryos which derived from matured GV oocytes were developed to blastocyst.

**Conclusion:** In the present study, GV oocytes have a similar maturation rate but higher fertilization and cleavage rate compared with MI oocytes. This finding indicates that if GV oocytes matured they fertilized and cleaved better than MI oocytes.

**Key words:** Human oocyte, IVM, Fertilization, Embryo.

## O-5

### Effects of different doses of ethanol on sperm parameters, chromatin structure and apoptosis in adult mice

Rahimipour M<sup>1</sup>, Talebi AR<sup>2</sup>, Anvari M<sup>2</sup>, Abbasi Sarcheshmeh A<sup>1</sup>, Omid M<sup>1</sup>.

1. Department of Biology and Anatomical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Andrology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: prof\_talebi@ssu.ac.ir

**Introduction:** Chronic ethanol abuse causes reproductive organ failure, as well as infertility in both human and laboratory animals.

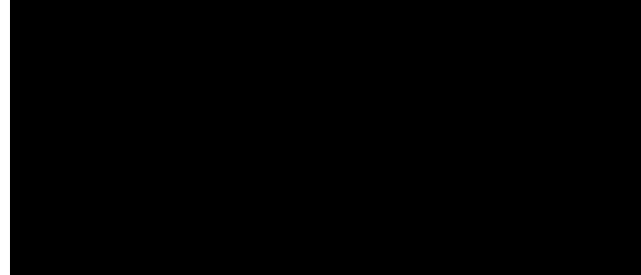
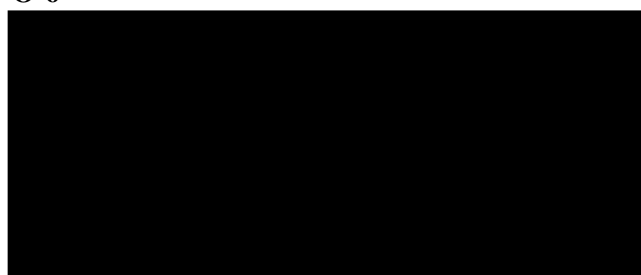
**Materials and Methods:** Totally 36 adult male mice were equally divided into 4 groups. Group 1 received ethanol (10% v/v) containing saccharin (0.2% w/v), group 2 received ethanol (5% v/v) containing saccharin (0.1% w/v), group 3 was treated with saccharin (0.2% w/v) and group 4 served as control and fed on basal diet for 35 days. Finally, left cauda epididymis of each animal was cut and placed in Ham's F10 medium. Retrieved spermatozoa were used to analyze count, motility, morphology and viability. Sperm chromatin condensation and DNA integrity were assessed by five different tests including chromomycin A3 (CMA3), toluidine blue (TB), sodium dodecyl sulfate (SDS), and SCD (sperm chromatin dispersion) and sperm apoptosis was assessed by TUNEL.

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

**Conclusion:** The results showed that the alcohol has negative effects on sperm parameters, chromatin/DNA integrity and apoptosis in mice. On the other hand, it should be noted that these alcohol-induced sperm anomalies may be dose-dependent.

**Key words:** Ethanol, Sperm, Chromatin, Apoptosis, Mice.

## O-6



## O-7

### Evaluating effects of the OCT4B1 spliced variant knocking down and over-expression on stem and cancer cell lines

Farashahi Yazd E<sup>1,2</sup>, Mowla SJ<sup>1</sup>.

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
2. Department of Genetics, Medical School, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: ehsanfarashahi@ssu.ac.ir

**Introduction:** OCT4 is one of the most critical and important genes in self-renewality and pluripotency of cells. The expression of this gene has been detected in stem cells and tumoral samples of a variety of cancers. This gene possesses three spliced variants termed OCT4A, OCT4B and OCT4B1. OCT4A is the variant of greatest importance playing the dominant role in pluripotency and self-renewality of stem cells. The role of the OCT4B variant has not been obviously determined and OCT4B1 is a recently-found spliced variant of OCT4 bearing substantial resemblance to OCT4B. This variant exhibits the expression pattern similar to OCT4A in embryonic stem cells in a manner that its expression dramatically declines during differentiation.

**Materials and Methods:** In order to gain insight into the biological role and function of this variant, over-express and suppression of the variant was investigated. We have designed specific siRNAs to suppress OCT4B1 expression in stem and tumor cell lines. We have also constructed an HA-tagged OCT4B1, which generates an N-terminal tagged protein, detectable by HA antibody. The transduced cells (5637 and USSC) then were lysed and the expression of HA-OCT4B1 transcript and protein detected by means of real-time PCR and Western Blotting. The apoptosis rate and cell viability of these cells were also investigated by flowcytometry after OCT4B1 suppression and over-expression.

**Results:** The results revealed splicing of most OCT4B1 pre-spliced molecules to OCT4B, but with varying degrees of splicing in different cells and various conditions, cells are exposed to including heat and genotoxic stress. Under heat stress, the ratio of OCT4B1/OCT4B is elevated, however in genotoxic stress the converse occurs. This ratio alteration is also observed at the level of protein expression. The generated OCT4B1 protein is a truncated and stable protein with an approximate molecular weight of 14 KDa consistent with the predicted ORF. The cellular examination of OCT4B1 over-expression in 5637 cells under normal condition suggested the increase of cell population in G1 phase and the augmentation of cell resistance against heat shock-induced apoptosis. The results obtained through suppression of OCT4B1 in 5637 and A549 cells demonstrated the rise of apoptosis to 31% and 13% in these cells, respectively. However, the molecular study implied the suppression of OCT4B1 gave rise to a remarkable reduction of OCT4B

expression.

**Conclusion:** Taken together, our findings reveal novel insights into the biological function of OCT4B1 in heat and genotoxic stress and resistance against apoptosis, although questions abound on the molecular mechanisms connecting the OCT4B1 variant and its biological role(s) in the context of stress condition which need to be answered.

**Key words:** OCT4B1, Knocking down, Over-expression, Stem cell, Cancer.

## O-8

### In vitro differentiation of spermatogonia cells into oligodendrocyte like cells and transplantation into demyelination model

Nazm Bojnordi M<sup>1</sup>, Movahedin M<sup>1</sup>, Tiraihi T<sup>1</sup>, Javan M<sup>2</sup>.

1. Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
2. Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Email: bojnordi@modares.ac.ir

**Introduction:** Embryonic Stem like cells (ES like cells) derived culturing of spermatogonia cells which has self-renewal and differentiation capacity to all three germ layers make them as a new and unlimited source for cell therapy and repair of neurodegenerative diseases.

**Materials and Methods:** In present study spermatogonia cells differentiated to oligodendrocyte like cells were transplanted to demyelination model. Spermatogonia cells were collected from neonatal mouse testis via a two-step enzymatic digestion. The spermatogonia cells were cultured in vitro and ES like cells colonies was appeared within 3 weeks. Real time PCR was performed to analyze the expression of pluripotency and spermatogonia specific genes. The pluripotency markers; SSEA1, SOX2 and Oct4 were evaluated as pluripotency markers using immunostaining and flowcytometry techniques. ES like cells were differentiated to neuroprogenitor cells and oligodendrocyte like cells and were transplanted to demyelination model rats. Cell integration and demyelination extension and intensity changes were evaluated using histological studies and immunocytochemistry.

**Results:** The pluripotency characteristic of ES like cells were confirmed by expression of the pluripotency genes; Nanog and c-myc and pluripotency markers SSEA-1, SOX2 and Oct4. Investigation of Nestin, NF68, Olig2 and NG2 by immunocytochemical and real time PCR studies indicated the differentiation of ES like cells to neuroprogenitor and oligodendrocyte like cells. Histological findings showed a significant decrease in demyelination extension and a significant increase in (re) myelination intensity in cell transplanted groups. Also on the base of PLP expression differentiation of transplanted cells was confirmed to myelinogenic cells using immunocytochemistry technique.

**Conclusion:** ES like cells derived from spermatogonia cells can differentiated to neuroprogenitor and oligodendrocyte like cells that can form myelin after transplantation into the demyelination model in rat.

**Key words:** Spermatogonia cells, ES like cells,

Neuroprogenitor cells, Oligodendrocyte, Demyelination.

## O-9

### Sarcotoxin Pd, a new antimicrobial and spermicidal peptide from the insect *Paederus dermatitis*

Zare-Zardini H<sup>1</sup>, Fasahat F<sup>2</sup>, Halvaei I<sup>2</sup>, Nabi A<sup>2</sup>.

1. Department of Pediatric, Hematology, Oncology and Genetics Research Center, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: farzaneh.fasahat@gmail.com

**Introduction:** Sexually transmitted infections and unplanned pregnancies are two great concerns in the reproductive health. Different agents are used for male contraception but it is necessary to introduce other compounds that have antimicrobial properties in order to reduction of sexually transmitted diseases as well as male contraception. The aim of this study was to purify new spermicidal peptides with antimicrobial properties from *Paederus dermatitis* (Pd).

**Materials and Methods:** The peptide purification was done with gel filtration and Reverse-Phase High Performance Liquid Chromatography (HPLC). The sequencing was carried out by Mass/Mass. The antimicrobial activity was tested by Radial Diffusion Assay (RDA) and Minimal Inhibitory Concentration (MIC) methods. After preparation of normozoospermic specimens, different doses of 3, 7.5, 15, 30 and 125 µg/ml were tested at different time intervals (0.3, 5, 10, 15 min). Eosin staining method was applied for sperm viability detection.

**Results:** The results showed that the sarcotoxin Pd showed considerable antimicrobial activity against Gram positive and Gram negative bacteria [minimum inhibitory concentrations (MIC), 6.12-19.24 µg/ml] as well as fungi (MIC, 18.62 to 25.26 µg/ml). In addition, this new peptide showed no hemolytic activity against human red blood cell. This peptide is composed of 34 amino acids and its sequence is: GWLKKIGKKIE RVGQHTRGLGIAQIAANVAATA. Total spermicidal activity was shown after addition of 125 µg/ml for 0.3 min.

**Conclusion:** Preliminary results showed potential spermicidal as well as antimicrobial activities of *sarcotoxin Pd*. It seems this peptide is potential candidate in order to use in male contraception which can be used as a spermicidal agent in male condoms.

**Key words:** Antimicrobial, *Paederus dermatitis*, Sexually transmitted infections, Spermicidal, unplanned pregnancies.

## O-10

### Immunological evaluation and comparison of normal and endometriosis menstrual blood stromal stem cell

Nikoo S<sup>1</sup>, Ebtekar M<sup>2</sup>, Jeddi-Tehrani M<sup>3</sup>, Zarnani AH<sup>4,5</sup>.

1. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
  2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
  3. Monoclonal Antibody Research Center, Avicenna Research Institute, Tehran, Iran.
  4. Nanobiotechnology Research Center, Avicenna Research Institute, Tehran, Iran
  5. Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
- Email: zarnani@avicenna.ac.ir

**Introduction:** Endometriosis is known as the existence of endometrial cells and tissues outside the uterine cavity. Retrograde reflux of menstrual cells during menstruation is considered as the dominant theory for the development of endometriosis. In this regard, menstrual blood stromal stem cells (MensSCs) might be involved in the pathogenesis of endometriosis. Additionally, several defects in immune responses and especially lymphocyte function have been proposed to contribute to implantation of menstrual blood cells in peritoneal cavity and development of endometriosis.

**Materials and Methods:** Here, we compared normal and endometriosis MensSCs with respect to their morphology immunophenotype, ability to express indoleamine 2,3 dioxygenase (IDO) enzyme, cytokines production, invasion and adhesion capacity; MensSCs were obtained from menstrual blood of normal and endometriosis individuals and then their immunophenotype were determined by flowcytometry. Morphological comparison between endometriosis and normal MensSCs was explored through culture in matrigel-coated plates. In addition, MensSCs of both groups were cocultured with allogenic mononuclear cells in the transwell culture system. After 3 days, IDO expression in MensSCs was evaluated by real-time PCR and western blot. Production of cytokines in the coculture also was measured by ELISA. Moreover, the invasion and adhesion potential of endometriosis and normal MensSCs was compared.

**Results:** Endometriosis MensSC showed significantly higher expression of CD9, CD29 and CD10 in comparison with normal MensSCs (p<0.05). In addition they showed different morphological patterns in three-dimensional culture. IDO-expression was significantly higher in endometriosis MensSCs a finding that was in line with the higher amounts of IFN-γ percent in endometriosis MensSCs coculture system. Furthermore, endometriosis MensSCs had more invasive potential compared to the normal group, while no significant difference was observed regarding the adhesion capacity.

**Conclusion:** Endometriosis MensSCs are different from normal ones with respect to their phenotypic and functional characteristics. Bearing in mind all the observed differences, endometriosis MensSC could be considered to play a major role in the pathogenesis of endometriosis.



**Key words:** Endometriosis, Menstrual blood, Stem cells, indoleamine 2, 3 dioxygenase (IDO) enzyme.

## O-11

### Lithium induced dysfunction in the rat ovary: involves inhibition of angiogenesis in the corpus luteum

Khodadadi M<sup>1</sup>, Ansari pirsaraie Z<sup>2</sup>.

1. Department of Studies in Zoology, University of Mysore, Mysore, India.
2. Animal Sciences Department, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

Email: khodadadi\_mail@yahoo.in

**Introduction:** Progesterone production by the ovary is dependent on the key proteins involved in the steroidogenesis, and also vascularisation of the corpus luteum (CL). We have recently shown that ovarian steroidogenesis is affected by lithium chloride (LiCl) treatment, an effective drug for the treatment of bipolar disorder in gonadotropin-stimulated immature rat. Objective: In this study, we have investigated whether reproductive toxicity of lithium is associated with alterations in the expression of vascular endothelial growth factor (VEGF) and its receptor, the primary mechanism of CL angiogenesis control. Materials and Methods: Immature 35- day- old Wistar rats were injected with LiCl (2.0 mg/kg/ day i.p.) or distilled water (0.5 ml) for 15 days. Then, all rats were given single injection of pregnant mare's serum gonadotrophin (PMSG) on the 13th day of experiment and followed by single injection of human chorionic gonadotropin (hCG) 48 hours later. The last injection of LiCl was given 12 hours post-hCG injection. Rats injected only with distilled water and gonadotropins were served as control group. Blood and ovaries were collected at 4-hours interval from 8 to 24 hours post-hCG injection. Serum levels of progesterone were measured by ELISA and CL formation was determined by histological analysis. Then the VEGF and KDR genes expression were examined using real-time polymerase chain reaction (RT-PCR).

**Results:** Results showed serum levels of progesterone and transcript levels of VEGF were markedly decreased in the gonadotropin-stimulated rats following LiCl treatment.

**Conclusion:** It is concluded that vessels formation and critical step of angiogenesis were affected by LiCl in gonadotropin-stimulated rat ovary. This study has provided evidences that LiCl is an effective factor for suppressing of angiogenesis genes expression in the rat ovary.

**Key words:** Angiogenesis, Progesterone, Lithium.

## O-12

### TLRs expression in follicular cells of infertile PCOS women

Taghavi SA<sup>1</sup>, Ashrafi M<sup>2, 3</sup>, Mehdizadeh M<sup>1, 4</sup>, Karimian L<sup>5</sup>, Joghataie M<sup>1, 4</sup>, Aflatoonian R<sup>3</sup>.

1. Department of Anatomy, School of Medicine, Tehran

University of Medical Sciences, Tehran, Iran.

2. Department of Obstetrics and Gynecology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
3. Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
5. Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: R.Aflatoonian@gmail.com; m-Ashrafi@sina.tums.ac.ir

**Introduction:** Polycystic ovary syndrome (PCOS) is a major endocrinopathy that occurs in approximately 5 - 25 % of women of reproductive age. Major underlying problems with PCOS are hormonal imbalance and anovulation. Emerging evidence demonstrates that PCOS is associated with systemic inflammation. Inflammation is result of stimulation of innate immunity. The main compartment of innate immunity is pattern recognition receptors (PRRs). The most important group of PRRs which also identified in female reproductive tract is Toll like receptors (TLRs) family. To date, TLR1-10 are characterized in human.

**Materials and Methods:** To investigate TLR1-10 gene expression in follicular cells obtained from PCOS women in compare to normal women. All procedures were approved by the Royan Ethics committee and informed consent was obtained prior to the collection of samples. Forty patients (20 infertile PCOS patients and 20 normal women with male factor infertility) underwent controlled ovarian stimulation. The follicular fluid was obtained from the largest follicle (>18 mm) then transferred to a sterile Petri dish. After oocytes removal, the fluid was centrifuged at 300g for 5 min. The supernatant was removed. Total RNA was extracted separately from cellular pallet in each group and real time PCR was performed. Differences in normalized expression values between samples were tested for significance using t test statistical analysis.

**Results:** The results were expressed as mean±SEM. The level of statistical significance was set at p<0.05. The mean relative expression of all TLR1-6, TLR8 and TLR9 genes was significantly higher in patients with PCOS in compare to normal women. TLR10 gene expression was significantly lower in PCOS than control. The expression of TLR7 genes revealed no significant difference in both groups.

**Conclusion:** Our findings suggested that TLRs are involved in pathophysiology of PCOS.

**Key words:** Follicular cells, Innate immunity, PCOS, TLR.

## O-13

### Evaluation of anti-tumor and anti-inflammatory effects of vitamin D<sub>3</sub> in endometrioma

Delbandi AA<sup>1</sup>, Mahmoudi M<sup>1</sup>, Zarnani AH<sup>2, 3</sup>.

1. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

2. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: zarnani@avicenna.ac.ir

**Introduction:** Endometriosis is a chronic inflammatory disease characterized by the growth of endometrial tissue outside the uterine cavity. In patients with ovarian endometriosis (endometrioma), the risk of ovarian cancer increases fivefold. Regarding its anti-tumor and anti-inflammatory action, the effect of vitamin D<sub>3</sub> on pre-cancerous state of and anti- and pro-inflammatory cytokines production by endometrioma cells were investigated in this study.

**Materials and Methods:** Stromal cells were prepared through enzymatic digestion of eutopic and ectopic endometrial tissues from 13 endometriotic patients. Endometrial stromal cells of 10 non-endometriotic patients served as control. Following cell characterization through immunocytochemistry and flowcytometry, stromal cells were cultured in the presence or absence of the active form of vitamin D<sub>3</sub>. Cultured cells were analyzed for proliferation, adhesion to extracellular matrix and invasion to matrigel. Additionally, the levels of IL-6, IL-8, IL-17, TGF- $\beta$ , TNF- $\alpha$  and IFN- $\gamma$  in culture supernatants was determined using ELISA.

**Results:** In all groups, vitamin D<sub>3</sub> treatment resulted in a significant increase of attachment ( $p < 0.01$ ), while decreased cell invasion to matrigel ( $p < 0.05$ ) and proliferation capacity ( $p < 0.01$ ). Ectopic and eutopic endometrial cells from patients secreted higher levels of IL-6 and IL-8 compared to the control group in the absence of vitamin D<sub>3</sub> ( $p < 0.05$ ), whereas, vitamin D<sub>3</sub> treatment resulted in a significant decrease in IL-6 production by patient ectopic cells ( $p < 0.05$ ). Stromal cells from all groups, showed no detectable secretion of other cytokines.

**Conclusion:** With regard to the reduced invasiveness and proliferative capacity and increased adhesion of stromal cells in the presence of vitamin D<sub>3</sub>, it seems that this hormone can effectively be used in inhibition of disease spreading or reducing cancer risk. The decreased production of IL-6, as a pro-inflammatory cytokine, by ectopic stromal cells following vitamin D<sub>3</sub> treatment implies anti-inflammatory action of this hormone which could be viewed as its potential beneficial effect over disease course.

**Key words:** Vitamin D<sub>3</sub>, Endometriosis, Inflammation, Ovarian cancer.

## O-14

### Human ovarian tissue vitrification as an alternative method for assisted reproduction

Abdollahi M<sup>1</sup>, Salehnia M<sup>1</sup>, Salehpour S<sup>2</sup>.

1. Department of Anatomical Sciences, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.
2. Department of Obstetrics and Gynecology, Medical Director IVF Center, Shahid Beheshti University of Medical Sciences, Ayatollah Taleghani Hospital, Tehran, Iran.

Email: abdollahi\_masume@yahoo.com; m.abdollahi@modares.ac.ir

**Introduction:** One of the new emerging techniques for fertility preservation in cancer patients is cryopreservation of ovarian fragments prior to medical treatment. The aim of this study is investigation of apoptosis in cultured human ovarian tissue after vitrification and warming in culture media.

**Materials and Methods:** Human ovarian tissue was cut into small pieces. Some pieces were vitrified and then warmed. Non-vitrified and vitrified samples were cultured for 14 days. Apoptosis assessments were done by morphological evaluation, DNA Laddering and TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay. Estradiol hormones were measured days 2 and 14.

**Results:** The normality rates of follicles in control and vitrified samples were 93.75% and 90.88% respectively. At the end of culture period 82.35%, 79% of follicles remained morphologically normal. There were no significant differences between these groups. The estradiol concentrations were increased from day 2 to day 14 of culture period ( $p < 0.05$ ). No patterns of DNA laddering were seen in first day and after 14 days in vitro culture of both non-vitrified and vitrified samples. Some TUNEL positive cells were seen in vitrified samples after 14 days culture in comparison with control group. There was no significant difference between the control and vitrified samples.

**Conclusion:** The results obtained by vitrification of ovarian tissue with this protocol were compatible with those of the fresh ovarian tissue.

**Key words:** Cryobanking, Vitrification, Human ovarian tissue, Apoptosis.

## O-15

### Prevalence of pericentric inversion 9, inv (9) (p11q12 or 13) in patients referred for different genetic conditions including recurrent abortions and infertility to a maternity hospital in Tehran

Behjati F, Bagherizadeh E, Zarbati N, Abdi A, Shajarepour L, Galabeigi Sh.

Department of Medical Genetics, Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran.

Email: fbehjati@gmail.com

**Introduction:** Pericentric inversion of chromosome 9, inv (9) (p11q12 or 13) is a balanced chromosome rearrangement with a frequency of about one per cent in the general population. This inversion when found in patients referred for recurrent abortions or infertility can be a challenge for genetic counseling. Although pericentric inversion 9 is usually regarded as a normal population variant, some genetic reports regard it as the cause for recurrent abortions or infertility.

**Materials and Methods:** This report investigates the contribution of inv (9) (p11q12 or 13) to different human genetic conditions. A total number of 3267 patients were referred for post natal chromosomal investigation. Referral reasons included recurrent abortions, infertility, ART failure, complex consanguineous marriage, anxiety and other conditions.

Herparnised peripheral blood was obtained. Cytogenetic analysis was carried out using standard techniques. GTG high resolution banding technique was used. 15-50 metaphase spreads were studied. In the case of mosaicism up to 100 metaphase spreads were examined.

**Results:** The prevalence of pericentric inversion, inv (9) (p11q12or13) amongst the patients was as follows: 21 out of 1230 (1.7%) patients with recurrent abortions, 9 out of 570 (1.58%) amongst infertile patients, 1 out of 198 (0.5%) amongst patients with ART failure, 7 out of 439 (1.6%) amongst patients with complex consanguineous marriage and anxiety, and 12 out of 830 (1.5%) amongst patients with mental disability and other genetic conditions.

**Conclusion:** Our data does not show a significant difference in the prevalence of pericentric inversion 9 amongst patients with recurrent abortions, infertility, consanguineous marriage, and other genetic conditions. However patients with recurrent abortions had slight increase (1.7%) of inv (9) (p11q12or13).

**Key words:** Pericentric inversion 9, Recurrent abortions, Infertility, Consanguineous marriage.

#### O-16

##### Report of cytogenetic results of prenatal diagnosis using amniotic fluid on referred patients to a maternity hospital in Tehran

Behjati F, Bagherizadeh I, Mohammadkhani N, Asami M, Hadipour F, Hadipour Z.

Department of Medical Genetics, Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran.

Email: fbehjati@gmail.com

**Introduction:** Prenatal diagnosis (PND) using amniocentesis for the detection of chromosome abnormalities is a gold standard test. It helps to prevent the birth of a child with genetic conditions, reducing burdens on the family and society. PND program in Iran is expanding, although it is mostly concentrated in Tehran. The objective was to carry out chromosomal analysis on amnion cells in fetuses referred PND.

**Materials and Methods:** A total number of 2500 women were referred to the Cytogenetics laboratory of Sarem Women's Hospital, Tehran between 2006-2012. The women were in the age range of 16-53 years and the gestational ages were between 13-32 weeks; mean age of women and gestational age was 32 years and 16 weeks respectively. The referral reasons were abnormal maternal serum screening test (61.1%), raised maternal age (22%), abnormal sonography (5.1%), parents with chromosome abnormality (1.5%), anxiety (1.3%), family history of abnormal child, abortion and stillbirths (6.2%), exposure to teratogene (0.3%), and other reasons (2.5%). Three cultures were processed using standard protocols. Chromosome analysis using GTG banding technique was carried out.

**Results:** The total chromosome abnormality rate was 5.5%. The aneuploidy rate was 3.8%. 1.6% had structural abnormalities. The rate of marker chromosome, balanced reciprocal translocations, and derivative

chromosome were 0.4%, 0.9% and 0.3% respectively. 1.2% of fetuses had pericentric inversion of chromosome 9. The rate of chromosome abnormality in each of the referral category is: 5.2% for abnormal maternal serum screening test, 5% for raised maternal age, 7.5% for abnormal sonography, 28.5% for parents with chromosome abnormality, 5.3% for family history of abnormal child, abortion and stillbirths, 0% for exposure to teratogens and anxiety and 3.3% for other reasons. Repeat samples were requested for 0.4% women because of culture failures.

**Conclusion:** Our findings were very helpful to the couple for the management of their fetus.

**Key words:** Prenatal diagnosis, Amniotic fluid, Chromosome abnormality, Maternal serum screening test.

#### O-17

##### Screening for chromosome abnormalities by microscopic chromosome analysis: A report of 2 years study of cytogenetic results of referred candidate for prenatal diagnosis

Dehghani MR<sup>1,2</sup>, Arrigo G<sup>1</sup>, Hashemian Z<sup>3</sup>, Zuffardi O<sup>1</sup>.

1. Department of Molecular Medicine, Section of Molecular Biology and Medical Genetics, University of Pavia, Italy.

2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

3. Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: dehghani.dr@gmail.com

**Introduction:** Screening for fetal chromosomal abnormalities is an essential part of prenatal care. Prenatal diagnosis enables early diagnosis of congenital anomalies and genetic disorders in utero, Microscopic chromosome analysis of cultured cells has been considered as the standard method for prenatal cytogenetic diagnosis since its first application to prenatal testing in 1966. It has become routine since the first use of chromosome banding (karyotyping) in the early 1970s. Karyotyping has proved to be highly reliable for the diagnosis of numerical chromosome abnormalities (aneuploidies) and large structural rearrangements [ $>5\text{-}10\text{Mb}$  pairs] in fetal cells obtained invasively by either amniocentesis in the second trimester of pregnancy or chorionic villus sampling in the first trimester. Advanced maternal age is the determinant risk factor in women older than 35 years. Therefore genetic counseling and amniocentesis and CVS is offered to these women.

**Materials and Methods:** In total 2974 women were referred to prenatal microscopic section of Pavia University during 2009 and 2010. Their gestational age was between 10-26 weeks (mean 15.3 weeks). The cultures were set up and processed using standard protocols. Chromosome analysis using QFQ banding technique was carried out. A minimum of 15 metaphase spreads using more than one culture were investigated.

**Results:** The referral indications were raised maternal age (70.4%), positive family history (3.2%), abnormal sonography (10.6%), abortion and stillbirths (3.9%),

abnormal combine test (4.9%) and others (4.9%). Chromosome analysis using QFQ revealed the following results: The total chromosome abnormality rate was 4.03%, the aneuploidy rate was 3.09% where trisomy 21 was the most common one (44.6%), and 0.40% of candidate had structural abnormalities where translocations were the most frequent in this category. The rate of chromosomal abnormality based on referral indications was as follows: advanced maternal age

1.68%, abnormal sonography 19.62%, abortion and stillbirths 25% and other indications 53.7%.

**Conclusion:** Based on our results and similar studies, chromosomal microscopic analysis like QFQ-banding and GTG-banding are reliable and convenience methods for detection of the most common chromosomal abnormalities in the first step of prenatal screening test.

**Key words:** Prenatal diagnosis, Chromosome abnormality, QFQ-banding.

## Poster Presentations

### P-1

#### Effect of progesterone supplementation on natural frozen-thawed embryo transfer cycles

Rahsepar M<sup>1</sup>, Rahmani E<sup>2</sup>, Eftekhari M<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Bushehr University of Medical Sciences, Bushehr, Iran.

Email: drrahsepar@hotmail.com

**Introduction:** The transfer of cryopreserved embryos can be timed with ovulation in a natural cycle or after artificially preparing the endometrium with exogenous hormones. Progesterone is essential for the secretory transformation of the endometrium that permits implantation as well as for the maintenance of early pregnancy. The purpose of this study is to assess the effect of luteal phase supplementation on pregnancy rates in natural.

**Materials and Methods:** The study was designed as a prospective randomized clinical trial of 102 women undergoing an embryo transfer in a natural cycle. The women in the interventional group (n=51) received intramuscular progesterone 50 mg twice a day starting from 36 hours after the HCG administration. The control group (n=51) did not receive any progesterone support.

**Results:** There were no significant differences of demographic characteristics between the groups and no statistically significant differences were observed between them in clinical pregnancy rate (33.3% vs 27.5%, p=0.66). There were no differences in implantation rate or spontaneous abortion rate either.

**Conclusion:** In conclusion, our results suggest that a luteal phase support does not affect clinical pregnancy rates in natural frozen-thawed embryo transfer cycles.

**Key words:** Progesterone, Pregnancy rate, Frozen embryo transfer, Natural cycle.

### P-2

#### Barriers of child adoption in infertile couples: Iranians' views

Bokaie M<sup>1, 2</sup>, Farajkhoda T<sup>1</sup>, Enjebaz B<sup>1</sup>, Heidari P<sup>1</sup>, Karimi-Zarchi M<sup>3</sup>.

1. Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Obstetrics and Gynecology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: mah\_bokaie@ssu.ac.ir

**Introduction:** There are many reasons why some couples do not become parents. This study was designed to determine barriers of child adoption in infertile couples in Iran.

**Materials and Methods:** This cross sectional study was carried out at Shahid Sadoughi University of Medical Sciences. The research program comprised consecutively in 240 infertile couples.

**Results:** Although 230 (96%) of the respondents had heard of child adoption, only 89 (37.3%) knew its correct meaning. Fifty-four (24%) women knew how to adopt a baby while the rest did not; 196 (82%) respondents expressed their unwillingness to adopt a baby while the remaining 44 (18%) were willing. Hope for childbearing (78%) was the main barrier to adopting a child.

**Conclusion:** The barriers mentioned were cultural practices, stigmatization, financial implications, and technical problems.

Most of the infertile Iranian couples will prefer to stay even so without children or to think about new treatment.

**Key words:** Child Adoption, Infertility, Barriers, Iranian views, Cultural and social barrier.

### P-3

#### The effects of calligonum extract on sperm parameters and expression of catsper gene in aging male mice

Askarijahromi M<sup>1</sup>, Movahedin M<sup>1</sup>, Mowla SJ<sup>2</sup>, Batooli H<sup>3</sup>.

1. Department of Anatomy, Tarbiat Modares University, Tehran, Iran.
2. Department of Genetics, Tarbiat Modares University, Tehran, Iran.
3. Isfahan Research Center of Agriculture and Natural resources, Kashan Botanical Garden, Kashan, Iran.

Email: mansoure@modares.ac.ir

**Introduction:** Catsper family code protein cation channels in plasma membrane of sperm. Catsper proteins are responsible for entering Ca<sub>2</sub><sup>+</sup> to the cell and play an important role in sperm motility and male fertility. Antioxidants are vital for sperm motility too. Calligonum extract possess some of the important antioxidant like catechin and quercetin.

**Materials and Methods:** Dose response was achieved in three doses (10 mg/kg, 30 mg/kg, 50 mg/kg). 5 mice in each group were considered and gotten injection for 5 weeks intraperitoneally (IP), after this period the mice were sacrificed and sperm parameters were analyzed. Does 30 mg/kg of calligonum extract was determined as optimum dose. Tunel staining test in these groups was achieved too. Fifteen aging male mice (11-13 months) were divided in to three groups: control, sham and experiment. The experiment groups were injected IP with calligonum extract weekly for up to 5 week. The sham groups were injected IP (DMSO). Sperm parameters were analyzed. Expression of Catsper genes was analyzed by real time PCR.

**Results:** Our results showed that after calligonum treatment, the sperm parameters were improved in 30 mg/kg group comparison with 10 mg/kg and 50 mg/kg groups (p<0.05). The results also demonstrated decreasing of cell apoptosis in 30 mg/kg group

comparison with the other group ( $p < 0.05$ ) in testis tissue. Our data showed that there was a statistical significance between the expression of catsper 2, 4 in aging experiment group comparison with aging control group ( $p < 0.05$ ).

**Conclusion:** We investigate that the calligonum extract (30 mg/kg) can improve sperm parameters and change the expression of catsper genes in aging male mice. This herbal extract can use as an antioxidant component for clinical usages

**Key words:** Spermatozoa, Calligonum extract, Catsper gene,  $Ca_2^+$  channels.

#### P-4

##### Domestic violence in Iran

Bokaie M<sup>1,2</sup>, Zare M<sup>1</sup>.

1. Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: mah\_bokaie@ssu.ac.ir

**Introduction:** Domestic violence refers to the physical, sexual or emotional abuse inflicted on a spouse by the other. The purpose of this article is to determine the prevalence of domestic violence in Iran.

**Materials and Methods:** In this review study, we analyzed more than 40 articles about domestic violence in Iran.

**Results:** The highest prevalence of physical violence was reported in Tehran (68%). Emotional violence was reported 87% in Amol and 87.3% in Tehran and sexual violence was highest in Takab (77%). In addition, verbal violence was seen in 74% cases in Takab. In Ardabil and Lorestan lowest violence were reported. Many of cases afraid to report domestic violence.

**Conclusion:** We recommend that systematic screening for domestic violence should be performed.

**Key words:** Domestic violence, Iran, Abuse.

#### P-5

##### Cancer screening in Iranian middle-age women: mixed method approach

Enjezab B<sup>1</sup>, Taleghani F<sup>2</sup>, Farajzadegan Z<sup>3</sup>, Aflatoonian A<sup>4</sup>.

1. Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Nursing, Nursing and Midwifery Care Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
3. Department of Community Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: taleghani@nm.mui.ac.ir

**Introduction:** Today cancer is one of the common causes of incidence and mortality in the world. Now, cancer is third cause of death in Iran and annually happened 30,000 cases of death in Iran. With starting early screening can be prevented than progressive cancers and mortality. In middle age increase incidence of many cancers including breast, cervical and colon.

**Materials and Methods:** The present studies investigated the amount of screening for cancer in middle-aged women and explain the factors. In the research 483 middle-age women was selected with cluster random sampling in the quantitative phase and 12 middle-age women was selected with purposive sampling in the qualitative phase. In the first stage data gathering was done with questionnaire and analyzed with descriptive statistics and in the second stage qualitative semi-structured interviews and data were analyzed with content analysis.

**Results:** The results showed that mean age of the participants were about 46 years old, the majority of women were housewives (85.1%), the majority of people (59.4%) have educated under diploma. 64%, 67.3%, 78.7%, 62.4% and 94.4% respectively of people never have not done BSE, CBE, mammograms, Pap test, fecal occult blood test. Qualitative research results also showed a lack of knowledge, the cost of the screening examinations, lack of financial independence of women and neglect their spouse, fear of cancer, embarrassment and myths of the main obstacles to cancer screening and knowledge, encourage of health professionals and observation of cancer in familiar persons were the most of the motives of cancer screening.

**Conclusion:** Considering the prevalence of cancers in this age group, it need proper planning to increase awareness and need to training for spouses and providing to screening services in the healthy policies.

**Key words:** Cancer screening, Papsmear, BSE, CBE, Mammography, Fecal occult blood test, Middle-age women.

#### P-6

##### Health promoting behaviors in a population-based sample of middle-aged women and its relevant factors in Yazd, Iran

Enjezab B<sup>1</sup>, Farajzadegan Z<sup>2</sup>, Taleghani F<sup>3</sup>, Aflatoonian A<sup>4</sup>, Bokaie M<sup>1</sup>.

1. Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Community Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
3. Department of Nursing, Nursing and Midwifery Care Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: enjezabbehnaz@yahoo.com

**Introduction:** This study is carried out with the aim of describing the pattern of health-promoting behaviors (HPBs) among middle-aged women and its relevant factors in Iran as a Muslim developing country.

**Materials and Methods:** In a cross-sectional study, 483 middle-aged women were selected using cluster random sampling from Yazd city in Central Iran. An adjusted Persian version of Health Promoting Lifestyle Profile II was used for measuring HPBs. The demographic data were collected too.

**Results:** The average score of middle-aged women HPBs was  $2.73 \pm 0.36$  ( $2.69 \pm 2.76$ , 95%). Spiritual growth and physical activity had the highest and the lowest score in HPBs' dimensions, respectively. HPBs had a positive significant correlation with age ( $p=0.02$ ) and education level ( $p=0.001$ ) and a negative significant correlation with number of children ( $p=0.005$ ). HPBs had a higher level in retired women than employees and housewives.

**Conclusion:** In spite of a desirable level of HPBs in spiritual growth and interpersonal relationship dimensions among this group, their level of HPBs in physical activity and health responsibility dimensions was undesirable. It means that the feature of health among Iranian middle-aged women in a Muslim developing country is different from the industrialized developed and also the non-Muslim countries; hence, they need a different intervention programs.

**Key words:** Health behavior, Health promotion, HPLP II, Iran, Middle age, Women.

## P-7

### Systematic review of epidemiology of gestational diabetes in Iran

Enjezab B<sup>1</sup>, Janghorbani M<sup>2</sup>.

1. Department of Nursing and Midwifery, Faculty of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Epidemiology and Biostatistics, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran.

Email: behnaz\_enjezab@ssu.ac.ir

**Introduction:** Although gestational diabetes is a well-known cause of pregnancy complications, its epidemiology in Iran has not been studied systematically. The aim of this systematic review of published data was to describe epidemiology of gestational diabetes in Iran.

**Materials and Methods:** We searched MEDLINE, www.sid.ir, www.magiran.com, www.iranmedex.com, and www.irandoc.ac.ir through March 2009, and examined the reference lists of pertinent articles, limited to studies in humans. The 20 eligible studies (4 English and 16 Farsi), included 20,985 participants and 826 incident cases of gestational diabetes.

**Results:** The prevalence of gestational diabetes was 3.9% (95% confidence interval 3.64-4.19). These findings were more consistent in Tehran studies, and its prevalence was 1.3-11.9% in other parts of the country.

We found that differences in screening programs and diagnostic criteria or various ethnic groups make it difficult to compare frequencies of gestational diabetes among various populations. Nevertheless, factors that place women at increase risk of gestational diabetes were age; body mass index and number of pregnancies. We conclude that the epidemiological data suggest that gestational diabetes is common among Iranian women. It is possible that improving insulin sensitivity with diet, exercise and drugs such as metformin may reduce the risk of gestational diabetes in women at high risk, such as women with polycystic ovary syndrome, impaired glucose tolerance, and a history of gestational diabetes.

**Conclusion:** Large studies are needed to clarify this issue and to develop appropriate diabetic prevention strategies that address the potentially modifiable risk factors.

**Key words:** Gestational diabetes, Epidemiology, Systematic review, Risk factors, Iran.

## P-8

### Predictive value of aspartate aminotransferase and alanine aminotransferase levels in vaginal fluid for the diagnosis of premature rupture of membranes

Asghrnia M, Dirbaz Z, Salamt F.

Reproductive Health Research Center, Alzahra Hospital, Guilan University of Medical Sciences, Rasht, Iran.

Email: maryamasgharnia@yahoo.com

**Introduction:** Premature rupture of membranes (PROM) occurs in 10-12% of pregnancies and 30-40% of preterm labors are related to PROM. Early diagnosis of PROM is very important due to its impact on pregnancy outcomes. High false positive and negative cases in Nitrazine and fern test lead to the measure of biochemical markers in vaginal fluid for the diagnosis of PROM. The aim of this study was to determine the diagnostic value of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in vaginal.

**Materials and Methods:** A total of 148 pregnant women were enrolled in the study. 74 patients were in PROM group and 74 in control group between the 26-36th gestational weeks. AST and ALT levels in vaginal fluid were measured in each group. Mann Whitney U-test was used to compare AST and ALT levels in each group. Receiver operating characteristic (ROC) curve was used to determine AST and ALT optimal cut points for the diagnosis of PROM.

**Results:** The mean of AST level in vaginal fluid was  $12.77 \pm 10.06$  in PROM group vs.  $6.91 \pm 10.92$  in control group ( $p<0.001$ ). While there were no significant difference between ALT levels in PROM group  $1.51 \pm 3.17$  and control group  $0.89 \pm 1.15$  ( $p=0.49$ ). There weren't significant differences between Background characteristics (mother age, gestational age, N. of parity) in PROM and control group too ( $p>0.05$ ). Optimal cut point of AST for the diagnosis of PROM

was 4.5 IU/L in this study. The sensitivity, specificity, positive and negative predictive values were 82.4%, 63.5%, 69.32%, 78.33% respectively.

**Conclusion:** According to the findings of this study, measurement of AST level in vaginal fluid can be used as a reliable test for early diagnosis of PROM, but there is no good cut point for ALT level that can be practically use.

**Key words:** Aspartate aminotransferase, Alanine aminotransferase, Premature rupture of membranes.

## P-9

### Impact of body mass index vs. physical activity and calorie intake on assisted reproduction

Ramezanzadeh F<sup>1</sup>, Kazemi A<sup>2</sup>, Nasr-Esfahani MH<sup>3</sup>.

1. Vali-e-Asr Reproductive Health Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.
2. Nursing and Midwifery Care Research Center, Faculty of Nursing and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran.
3. Department of Reproduction and Development, Reproductive Biomedicine Center, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran.

Email: kazemi@nm.mui.ac.ir

**Introduction:** The effect of BMI on reproductive outcome is complex. On the other hand, physical activity (PA) and calorie intake have been attributed to the regulation of endocrine and metabolic environment and body weight, which may represent an extreme endpoint of a more prevalent asymptomatic endocrine imbalance underlying ovulatory infertility.

**Materials and Methods:** This study was conducted to measure the effect of BMI considering the effect of calorie intake and physical activity (PA) on a prospective study was carried out on 236 infertile women who underwent IVF cycles. BMI, PA and calorie intake were assessed at study entry and association between these variables and assisted reproduction treatment were analyzed. Also based on BMI and PA, participants were divided into four groups (normal BMI/ inactive, normal BMI/ active, overweight/ inactive, overweight/ active). P-values of less than 0.05 were considered significant.

**Results:** Adjusted BMI for age, physical activity level, calorie intake level and etiology of infertility was not associated with the number of retrieved oocytes, fertilization rate, cleavage rate, number of embryos, number of high grade quality embryos and pregnancy rate. For women aged less than 36 the number of retrieved oocytes and embryos declined by increase in BMI independent of calorie intake and PA. The fertilization rate, cleavage rate, good quality number and pregnancy rate were not different. The retrieved oocytes were significantly higher in normal weight than in overweight women (active and inactive).

**Conclusion:** Age has a stronger negative effect on assisted reproduction parameters. Increased BMI, independent of calorie intake and PA, impairs the number of retrieved oocytes in women aged less than 36

but the success of the treatment cycle to access embryos of high grade quality is not affected.

**Key words:** Body mass index, Physical activity, Calorie intake, Assisted reproduction.

## P-10

### Correlation between history of Cu-IUD using and secondary infertility

Abdinasab M<sup>1</sup>, Dehghani Firouzabadi R<sup>1</sup>, Farajkhoda T<sup>2</sup>, Abdoli AM<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: farajkhoda\_t@yahoo.com

**Introduction:** Prevention of unwanted pregnancy and using appropriate type of contraception methods has been located in the core of women's reproductive health research. Intra Uterine Device (IUD) has been introduced as one of the most effective contraception method in worldwide but the relation between using of IUD and secondary infertility has not been well This study was conducted to determine correlation between history of Cu-IUD using and secondary infertility.

**Materials and Methods:** A case-control study was carried out from December 2010 to September 2011 in Fertility and Infertility Research Center of Yazd. 750 married women in reproductive age (15-45 years old) were selected as participants. They divided into two groups (case and control) based on previous history of inserting Cu T-IUD 380 A and were matched according age ( $\pm 2$  years). The inclusion criteria were length of IUD using at least for six month, without history of primary infertility or infertility treatments and without systematic diseases. Using of additional contraception method and occurrence of STD were determined as exclusion criteria. Data were gathered by structured questionnaire and were analyzed with X2 and Fisher-Exact tests.

**Results:** There were not any significant statistical differences in age, education level and occupation between case and control groups. The results showed that there was not any correlation between history of Cu T-IUD using and secondary infertility (3.5% in case group versus 2.7% in control group,  $p=0.63$ ).

**Conclusion:** This study confirmed safety of Cu T-IUD 380 A without any unpleasant and serious consequence such as infertility. So it could be used as an effective and safe contraceptive method.

**Key words:** Intra uterine device, Secondary infertility, Case control study.

## P-11

### Spermatogenesis assessment using testis specific gene expression

Eghbali M<sup>1</sup>, Modarressi MH<sup>1</sup>, Lakpour N<sup>2</sup>, Edalatkhah H<sup>3</sup>, Zeraati H<sup>4</sup>, Soltanghorae H<sup>3</sup>, Akhondi MM<sup>3</sup>, Sadeghi MR<sup>5</sup>.

1. Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.



2. Nanobiotechnology Research Center, Avicenna Research Institute (ARI), ACECR, Tehran, Iran.
3. Reproductive Biotechnology Research Center, Avicenna Research Institute (ARI), ACECR, Tehran, Iran.
4. Epidemiology and Biostatistics Department, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
5. Monoclonal Antibody Research Center, Avicenna Research Institute (ARI), ACECR, Tehran, Iran.

Email: sadeghi@avicenna.ac.ir; eghbalim@razi.tums.ac.ir

**Introduction:** In non-obstructive azoospermia (NOA) men, evaluation of spermatogenesis is performed with histopathological techniques as a gold standard. Pathological assessment of testes could be imprecise due to randomized biopsy and presence of focal spermatogenesis. Therefore, a sensitive and specific biomarker of sperm production in NOA testis is critical for future of male infertility treatment. NOA men showed a variety of defects in spermatogenesis stages, therefore molecular analysis of the stage-specific of gene expression in the testis could be confirmatory of histopathological techniques in evaluation of spermatogenesis in NOA men.

**Materials and Methods:** In this study, spermatogenesis status evaluated through expression of germ cell specific genes (DAZ, TSPY1, SPTRX3) and testicular biopsies were provided by infertile men undergoing diagnostic testicular biopsy in Avicenna infertility Clinic, Tehran. Histopathological evaluation was performed using H&E method. Semi-nested PCR was performed with designed specific primers. The molecular results were compared with the histopathological findings using Kappa test.

**Results:** Molecular and histopathological results were categorized in three groups: sertoli cell only syndrome, maturation arrest and hypospermatogenesis. RT-PCR results showed a significance difference (Kappa coefficient=0.009,  $p=0.894$ ) with the histopathological results. TSPY1, DAZ, SPTRX3 and SPTRX1 were expressed in 94%, 94%, 17.6% and 52.9% respectively in azoospermic men diagnosed as germ cell aplasia. This discrepancy is due to focal spermatogenesis in NOA testis.

**Conclusion:** Detection of DAZ, TSPY1 and SPTRX1 transcripts in testicular tissue can be used to predict the presence of mature spermatids/sperm in the testis especially in men diagnosed as spermatogenesis arrest using histopathological technique and may provide the better chance of finding the mature sperm to use through ART.

**Key words:** Male infertility, Non-obstructive azoospermia, Spermatogenesis, Germ cell and Testis specific Genes, Transcripts.

## P-12

### Morphine effects on folliculogenesis in culture medium and parthenogenesis

Arjmand Sh<sup>1</sup>, Behzadi M<sup>2</sup>, Amini S<sup>3</sup>, Emami Meybodi MA<sup>3</sup>, Nematollahi-mahani SN<sup>3</sup>.

1. School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

2. School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Anatomy, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

Email: lipahoc@gmail.com

**Introduction:** Morphine, a potent opioid alkaloid, has been used for more than a century as a strong anodyne and narcotic. It is over decades that morphine misuse has been common among some youth in many societies. Despite the vast conducted researches on how morphine affects the reproductive functions of the body, little is known about its effects on the process of folliculogenesis. Therefore, the mentioned deficiency made conductors' minds up to design a study in order to examine the rudimentary changes on the quality of folliculogenesis exerted by morphine.

**Materials and Methods:** To achieve the considered goal, the mice were addicted by oral morphine consumption. In the next step, 10 iu of PMSG was injected intraperitoneally to induce superovulation and after 48 hours the mice were sacrificed, the ovaries were weighted and then fixed in Boin's solution for a week. Afterward, the ovaries were sectioned into 5  $\mu$  segments and stained by H&E method. Thenceforth, the volume of ovaries was calculated by Cavalieri equation and not only was the number of small, growing, antral, and atretic follicles counted, but also the size of oocytes was measured by calibrated ocular lens.

**Results:** The analyses of the results were indicative of relative increase in the both size and volume of ovaries. Moreover, it depicted relative disorder in folliculogenesis progression, qua the percentage of small follicles had significantly increased in the treatment group comparing to the control one ( $p<0.001$ ), whereas the rate of atretic follicles had remarkably decreased with the same p-value. No significant difference was observed in the rest kind of follicles through treatment group versus the control.

**Conclusion:** In sum, it seems that oral morphine administration alters folliculogenesis in mice especially at transformation stage of small to growing follicles.

**Key words:** Morphine, Folliculogenesis, In vitro, Mice.

## P-13

### Derivation of ES-like cell from neonatal mouse testis in a novel culture condition

Javanmardi S<sup>1</sup>, Asadi MH<sup>1</sup>, Movahedin M<sup>2</sup>.

1. Department of Anatomical Sciences, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Department of Anatomical Sciences, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran.

Email: Asadi\_mh@yahoo.com

**Introduction:** Spermatogonial stem cell (SSC) is a self-renewing population of male adult stem cell. SSCs have differentiation potential similar to embryonic stem cells. These ES-like cells can be a potential source for pluripotent cells for stem cell-based therapy.

**Materials and Methods:** Using of an economical and

simple co-culture system for ES-like cells generation from neonatal mouse testis. Isolated testicular cells were cultured in DMEM/F12. Characteristics of the isolated cells and obtained ES-like cell were immunocytochemically confirmed by examining for the presence of PLZF, vimentin, Oct4 and Nanog protein. Expression of pluripotency and germ-cell specific genes, were analyzed by qPCR in derived ES-like colony and SSCs respectively.

**Results:** This experiment results indicated that our method of obtaining pluripotent ES-like cells from SSCs is simpler than the described methods. ES-like cells were immunopositive for pluripotency markers. ES-like cell qPCR results indicated significant increase in pluripotency genes expression and significant decrease in germ cell-specie genes expression.

**Conclusion:** We successfully established an easy and feasible method of neonatal mouse SSC derived ES-like cell in vitro culture. Apparently, an effective culture system would be a very valuable means for the mechanism studies of SSC self-renewal and ES-like cells generation.

**Key words:** Spermatogonial stem cell, ES-like cell, Pluripotency, Co-culture.

#### P-14

#### Effects of magnetized water on fertility and height of epithelial cells in pre-implantation stage

Hafizi L<sup>1</sup>, Gholizadeh M<sup>2</sup>, Ghorbani S<sup>3</sup>, Kazemi MR<sup>3</sup>, Hosseini G<sup>1</sup>, Mostafavi-Toroghi H<sup>4</sup>, Emami Meibodi N<sup>5</sup>, Haddadi M<sup>6</sup>, Karimi M<sup>3</sup>.

1. Department of Gynecology and Obstetrics, Imam Reza Teaching Hospital Medical Center, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.
2. Department of Chemistry, Faculty of Basic Sciences, Ferdowsi University, Mashhad, Iran.
3. Faculty of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.
4. Biochemistry of Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.
5. Department of Biomedical Engineering, Engineering Faculty, Islamic Azad University, Mashhad, Iran.
6. Microbiology Department, Medicine Faculty, Zahedan University of Medical Sciences, Zahedan, Iran.

Email: hesam.mostafavi@gmail.com

**Introduction:** Magnetized water has made many improvements in industry, agriculture and medicine. However its utilization in medicine still remains controversial. In this study we aimed to investigate the effects of magnetized water on fertility and height of epithelial cells in pre- implantation stage endometrium and fallopian tube in female mice.

**Materials and Methods:** Eighty female NRMI mice were randomly divided into two groups: the control group drank no magnetized water and the experimental (case) group drank magnetized water for 2 weeks. Female mice were superovulated and mated with male mice. At pre-implantation time samples of endometrium

and fallopian tube were obtained and after processing, the height of epithelial cells was measured by light microscope equipped with cell measurement software.

**Results:** Data analysis showed a significant increase in the mean number of corpus lutea and the height of epithelial cells in fallopian tube comparing the case with the control group ( $p < 0.01$ ,  $p < 0.05$  respectively) whereas endometrium epithelial cells of the case group showed insignificant increase in height, in compare with the control group ( $p > 0.05$ ).

**Conclusion:** Our results suggest that magnetized water can improve fertility indices in mice by increasing the number of corpus lutea and the height of fallopian tube epithelial cells. Therefore magnetized water, along with other methods, can be used to increase the success rate of fertility especially in assisted reproductive technology.

**Key words:** Magnetized Water, Fertility, Mice, Fallopian tubes, Endometrium.

#### P-15

#### Maturation capacity and viability assessment of human immature oocytes after vitrification and invitro maturation

Yazdanpanah F<sup>1</sup>, Eftekhari M<sup>1</sup>, Khalili MA<sup>1</sup>, Karimi HA<sup>2</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biology, Islamic Azad University, Jahrom Branch, Jahrom, Iran.

Email: farideh.yazdanpanah@gmail.com

**Introduction:** In general, 15% of oocytes collected in assisted reproductive cycles are immature. These oocytes may be matured following IVM program. It is possible to cryopreserve the immature oocytes for further use in ART after application of IVM. The aim was to determine the maturation rate, morphology and viability of human immature oocytes after fresh IVM and vitrified IVM program.

**Materials and Methods:** 63 women who underwent controlled ovarian stimulation for ART were included at Yazd Research and Clinical Center for Infertility. The investigation took place over a period of 5 months in 2011. 103 immature oocytes were retrieved from these infertile women. The women aged between 18-43 years old. 53 immature oocytes were used for fresh group and 50 immature oocytes for vitrification group. The maturation medium was Ham's F10 supplemented with 0.75 IU FSH, 0.75 IU LH (Menogon) and 40% human follicular fluid (HFF). After 48h, maturation and morphology were assessed in fresh-IVM and vitrified-IVM oocytes. Also, viability was assessed using PI/Hoechst staining.

**Results:** Oocytes Maturation rate were reduced in vitrification group (56.0%), in comparison with fresh group (88.7%,  $p < 0.001$ ). Oocyte viability rate after staining were reduced in vitrification group (56.0%), in comparison with fresh group (86.8%,  $p < 0.007$ ).

**Conclusion:** Vitrification reduces both the maturation capacity and viability of human immature oocytes. It is

recommended to apply IVM on fresh immature oocytes, instead.

**Key words:** *In vitro* maturation, Oocyte, Vittrification, Maturation, Viability.

## P-16

### Comparison of mild and microdose GnRH agonist flare protocols on IVF outcome in poor responders

Mashayekhi M<sup>1</sup>, Karimzadeh MA<sup>2</sup>.

1. Department of Obstetrics and Gynecology, Arak University of Medical Sciences, Arak, Iran.
2. Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: Dr.Mashayekhy@yahoo.com

**Introduction:** The poor responder is defined as patient with low ovarian reserve. The response to controlled ovarian hyperstimulation (COH) is lower regarding estradiol (E<sub>2</sub>) level, number of obtained oocytes, and fertilization, implantation and pregnancy rates in patients with low ovarian reserve. Despite the progression in ART, the preferred protocol for poor responders is still controversial. Several strategies are available to improve ART cycles outcome in poor responders.

**Materials and Methods:** To compare the IVF outcome of clomiphene citrate/ gonadotropin/ antagonist (mild protocol) and microdose GnRH 159 poor responder patients were randomized and ovarian stimulation were performed with clomiphene citrate, gonadotropin and antagonist (group I) or microdose GnRH agonist flare (group II) protocols. Main outcomes was clinical pregnancy rate and secondary outcomes were doses of gonadotropin administration and duration of stimulation.

**Results:** There were no significant differences in age, causes of infertility, basal FSH, BMI, duration of infertility, E<sub>2</sub> level on the day of hCG injection in both groups. Although the cancellation, fertilization, and clinical pregnancy rates were similar in both groups, the endometrial thickness, number of retrieved oocytes, mature oocytes and implantation rate were significantly higher in mild protocol. The doses of gonadotropin administration and duration of stimulation were significantly lower in mild protocol.

**Conclusion:** We recommend mild protocol in ART cycles for poor responders based on the results regarding less doses of used gonadotropin and a shorter duration of stimulation.

**Key words:** *Poor responders, GnRH agonist, GnRH antagonist, Clomiphene citrate, IVF outcome.*

## P-17

### Animal models of human PCOS, valuable tools for the diagnosis and treatment

Naderi MM, Heidari B, Borjian S, Sarvari A, Behzadi B, Akhondi MM, Shirazi A.

Reproductive Biotechnology Research Center, Avicenna Research Institute (ACECR), Tehran, Iran.

Email: a.shirazi@avicenna.ac.ir

**Introduction:** Polycystic ovary syndrome (PCOS) is the most frequent female endocrine disorder affecting 5-10% of women, causing infertility due to dysfunctional follicular maturation and ovulation, distinctive multicystic ovaries and hyperandrogenism, together with metabolic abnormalities including obesity, hyperinsulinism, an increased risk of type 2 diabetes and cardiovascular disease. The etiology of PCOS is unclear and decisive clinical studies are limited by ethical and logistic constraints. Consequently treatment is palliative rather than curative with focus on symptomatic approaches.

**Materials and Methods:** Hence, a suitable animal model could provide a valuable means to study the pathogenesis of the characteristic reproductive. So far some animal models including monkey, sheep, hours, rat and mouse are produced by various methods such as chemically-induced models (letrozole, androgen "prenatal or prepubertal", DHEA, RU486 and estradiol treatment) or transgenic models (leptin/insulin receptor knockout and theca specific Esr1 knockout mice) to simulate the key features of human PCOS. The prenatally androgenised rhesus monkey displays many characteristics of the human condition, including hyperandrogenism, anovulation, polycystic ovaries, increased adiposity and insulin insensitivity. However, the high cost of non-human primate studies limits the practical utility of these large animal models.

**Results:** Rodent models, on the other hand, are inexpensive; provide well-characterized and stable genetic backgrounds readily accessible for targeted genetic manipulation. Recent rodent models are which display both reproductive and metabolic disturbances associated with human PCOS.

**Conclusion:** This review aims to evaluate advantages and disadvantages of animal models used for human PCOS in order to find the best methods of diagnosis and therapy using animal models.

**Key words:** *Animal model, PCOS, Diagnosis, Treatment.*

## P-18

### Evaluation of cumulus specific genes expression pattern during in vitro culture of preantral follicles

Fatehi R<sup>1</sup>, Ebrahimi B<sup>1</sup>, Shahhosseini M<sup>2</sup>, Farrokhi A<sup>3</sup>.

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: b.ebrahimi@royaninstitute.org

**Introduction:** Appropriate development and maturation of vitrified ovarian tissue derived preantral follicles may not be acquired completely because of the vitrification and in vitro culture procedure.

**Materials and Methods:** In this study, the functionality of preantral follicles that isolated from Needle Immersed (NIV) and Solid Surfaced (SSV) Ovaries of 13-day old NMRI mice were removed and randomly placed into control, needle immersed (NIV) and solid surface vitrification (SSV) groups. For vitrification, ovaries were first transferred into equilibration and vitrification medium then they immersed in liquid nitrogen after loading by acupuncture needle in NIV group or cooling on a pre cooled steel surface in SSV group. In all experimental groups, isolated medium-sized preantral follicles were cultured in vitro for 12 days. Afterwards, follicle survival, follicular growth rate and the expression rate of cumulus expansion genes (Has2 and Ptgs2) were evaluated after 24 hour, 6, 10 and 12 days of culture in all experimental groups.

**Results:** Follicular survival rate was significantly different between control and SSV group ( $94.09 \pm 1.42$  vs.  $63.14 \pm 11.51\%$ ) on 6<sup>th</sup>, and also between SSV with control and NIV group ( $62.67 \pm 9.27$  vs.  $93.16 \pm 1.78$  and  $85.27 \pm 3.7\%$ ) on 10<sup>th</sup> and ( $58.4 \pm 12.72$  vs.  $92.82 \pm 2.84$  and  $85.1 \pm 10.53\%$ ) on 12<sup>th</sup> days of culture. follicular growth rate pattern was similar and ascending in control and vitrification groups. The expression patterns of Has2 and Ptgs2 were similar in both vitrification and control groups and their difference was not recognized significant between mentioned experimental groups.

**Conclusion:** Ovarian tissue vitrification by NIV and SSV methods did not reveal any negative effect on the expression of cumulus expansion genes during IVC of preantral follicles and despite delayed cooling rate in SSV method in comparison with NIV method, they showed similar findings.

**Key words:** Pre-antral follicle, In vitro culture, Gene expression, Ovarian tissue, Vitrification.

## P-19

### The effect of folic acid and zinc sulfate on endocrine parameters and seminal antioxidant level, after varicocelelectomy

Azizollahi S<sup>1</sup>, Nematollahi-mahani SN<sup>1</sup>, Azizollahi Gh<sup>2, 3</sup>, Baneshi MR<sup>4</sup>, Safari Z<sup>5, 6</sup>.

1. Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
2. Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Urology, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
4. Research Center for Modeling in Health, Kerman University of Medical Sciences, Kerman, Iran.
5. Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran.
6. Department of Anatomy, Division of Reproductive Biology, Tehran University of Medical Sciences, Tehran, Iran.

**Email:** saeedazizollahi@yahoo.com; s\_azizollahi@razi.tums.ac.ir

**Introduction:** Varicocele is among the most common problems which may lead to male infertility. Spermatogenesis is impaired as a consequence of this vascular defect, through mechanisms that are not well

described. The present study aimed to evaluate serum hormonal level (inhibin B, FSH and testosterone) and seminal plasma participants were randomly allocated into four experimental groups.

**Materials and Methods:** Our randomization schedule was; zinc sulfate/folic acid (ZF), folic acid (FA), zincs sulfate (ZS), and placebo (PL). The patients underwent varicocelelectomy, before which a blood and semen sample was obtained and also three and six months after varicocelelectomy for evaluation of blood hormonal level (FSH, testosterone, inhibin b) and seminal oxidative stress status (Nitric Oxide, SOD, TAC). Patients in different groups took orally one capsule per day after dinner following varicocelelectomy for 6 month.

**Results:** A significant rise in peripheral blood inhibin B and seminal plasma (SOD) activity was detected in ZF group after 6 month.

**Conclusion:** The present clinical trial indicates change in hormonal status of varicocelelectomized patients following long term administration of zinc sulfate and folic acid.

**Key words:** Folic acid, Zinc sulfate, Varicocelelectomy.

## P-20

### Seminal inflammatory markers and semen parameters in Chlamydia trachomatis infected males of infertile couples from Iran

Dehghan Marvast L<sup>1</sup>, Talebi AR<sup>2</sup>, Ghasemzadeh J<sup>2</sup>, Pacey A<sup>1</sup>.

1. Department of Human Metabolism, Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

**Email:** laleh.dm236@gmail.com

**Introduction:** Chlamydia trachomatis stimulates both humoral and cell-mediated immune responses. White blood cells produce inflammatory cytokines in response to foreign antigens including pathogens and chronic inflammation. Given the knowledge that infections may play a causative role in male infertility and male factor is a cause of infertility in 50% of cases, the hypothesis that increasing interleukin levels in seminal plasma correlates negatively with semen parameters in infertile men was tested.

**Materials and Methods:** The impact of C. trachomatis DNA and antibodies in the male partners of infertile couples and related this to semen FVU samples were examined for C. trachomatis DNA using PCR. Serum samples from 250 men were examined for serum C. trachomatis IgG and IgA and IgM using an immunofluorescence assay. Semen samples were examined for semen analysis, TUNEL assay and interleukins. Sperm DNA fragmentation was assessed by the TUNEL assay and levels of ILs were determined by ELISA.

**Results:** 45 men were positive for C. trachomatis IgG, 3 for IgM but none were positive for IgA. Concentration of semen leucocytes were correlated with levels of IL-6

( $p=0.012$ ). IL-8 levels were negatively correlated with semen volume ( $p=0.013$ ) and positively correlated with male age ( $p=0.039$ ) and concentration of seminal leucocytes ( $p=0.001$ ). Semen pH and level of IL-6 was significantly higher in the IgG positive men ( $p=0.056$ ;  $p=0.055$ ) whereas semen volume was significantly lower ( $p=0.001$ ).

**Conclusion:** The results of this study indicate the seminal interleukin levels might be a sensitive and useful marker of silent infection/inflammation of the male genital tract. Since there is no correlation between male age and semen volume, reduced semen volume must be caused by accessory glands infection without damage to sperm or spermatogenesis.

**Key words:** Seminal inflammatory markers, Chlamydia trachomatis, Infertility.

## P-21

### Effects of zinc sulfate and folic acid co-administration on sperm parameters, protamine content and acrosomal integrity of varicocelectomized patients

Azizollahi Gh<sup>1,2</sup>, Azizollahi S<sup>3</sup>, Babaei H<sup>4</sup>, Kianinejad MA<sup>5</sup>, Baneshi MR<sup>6</sup>, Nematollahi-mahani SN<sup>7,8</sup>.

1. Department of Urology, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
2. Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Anatomy, Division of Reproductive Biology, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
5. Food and Drug Laboratory, Vice Chancellor of Food and Drug, Kerman University of Medical Sciences, Kerman, Iran.
6. Research Center for Modeling in Health, Kerman University of Medical Sciences, Kerman, Iran.
7. Department of Anatomy, Afzalipour School of Medicine, Kerman University of medical sciences, Kerman, Iran.
8. Afzal Resaerch Institute (NGO), Kerman, Iran.

**Email:** nnematollahi@kmu.ac.ir

**Introduction:** Varicocele, a vascular lesion of the pampiniform plexus in the spermatic cord caused by melancholic blood was first described by the French surgeon Ambroise Pare in the 16<sup>th</sup> century. However several possible mechanisms have been proposed, such as failure of testicular growth and development of testes, impairment of spermatogenesis due to increase in intra scrotal temperature, oxidative stress, and leydig cell dysfunction.

**Materials and Methods:** A prospective randomized controlled study was undertaken to investigate the effects of zinc sulfate, folic acid and zinc One hundred male subjects with palpable varicocele were included in the study and randomized into four groups. Subjects received zinc sulfate, follic acid, zinc sulfate/follic acid or placebo for 6 months. At least one semen sample was

obtained before surgery and 3 and 6 months after surgical repair. Semen samples were evaluated for number, motility, progression and morphology as well as chromatin content and acrosomal integrity.

**Results:** Most of the evaluated parameters showed a mild improvement after varicocelectomy in the placebo group. Interestingly, co-administration of zinc sulfate and folic acid improved most factors significantly. Folic acid administration but not zinc sulfate could increase sperm number ( $p<0.05$ ). Hence, zinc sulfate was better than folic acid when change in morphology was assessed ( $p<0.05$ ), and none of them was significantly effective in sperm motility. In zinc sulfate and follic acid groups, protamine content and halo formation rate significantly improved.

**Conclusion:** We may conclude that co-administration of zinc and folic acid significantly improved sperm parameters and increased varicocelectomy outcomes. So, medical treatment with compatible drugs after surgery might be advantageous for obtaining acceptable results.

**Key words:** Zinc sulfate, Folic acid, Protamine, Acrosomal integrity, Varicocelectomy.

## P-22

### Long term testicular torsion evaluation to establish azoospermic model of spermatogonial stem cell transplantation recipient

Azizollahi S<sup>1</sup>, Aflatoonian R<sup>1,2</sup>, Sedigi-Gilani MA<sup>3,4</sup>, Asghari-Jafarabadi M<sup>6</sup>, Koruji M<sup>1,5</sup>.

1. Department of Anatomical Sciences, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Urology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
5. Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.
6. Department of Statistics and Epidemiology, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran.

**Email:** skoruji@tums.ac.ir

**Introduction:** Testicular torsion is a urological emergency disorder in which one testicle gets twisted in the scrotum, subsequently cutting off its blood supply. An affected testicle tends toward ischemia and reproductive system dysfunction. The purposes of this study were to investigate the long-term effect of testicular torsion on sperm parameters; testis structure unilateral testicular torsion was created.

**Materials and Methods:** The animals were divided into two groups each containing 15 mice. They underwent 2 and 4 hours of unilateral testicular ischemia, respectively. All animals in this experiment

were aged matched. The experimental groups were studied 2, 4 and 10 weeks after testicular ischemia reperfusion. Moreover, the left testes and epididymides were removed for sperm analysis and for weight and histopathological evaluation. Finally isolated SSCs were transplanted in the testes that underwent 2 hours of ischemia reperfusion, two weeks post-surgery.

**Results:** All the investigated parameters demonstrated a sharp decline at 2, 4 and 10 weeks after testicular torsion, whereas 2-hour ischemia was found to be less injurious in testicular tissue structure. Two months after xenotransplantation, the transplanted cells were localized in the basal of the seminiferous tubules of the recipient ischemic testes.

**Conclusion:** Torsion can cause permanent azoospermia in mouse. Also testicular torsion 2 weeks after the 2 hours ischemia reperfusion may prove useful for recipient preparation For SSCs transplantation in mouse.

**Key words:** Testicular torsion, Spermatogonial stem cells, Transplantation model.

## P-23

### Psychological impact of gender infertility diagnoses between husbands' and wives' approach to infertility affect marital and sexual satisfaction

Vizheh M<sup>1</sup>, Pakgozar M<sup>2</sup>.

1. Faculty of Midwifery, Islamic Azad University, Branch of Mahabad, Mahabad, West Azerbaijan, Iran.
2. Department of Reproductive Health, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran.

Email: maryamvizheh@yahoo.com

**Introduction:** Infertility has a deleterious impact on marital relationships. The effect of a gender-specific infertility diagnosis on the response of husbands versus wives has been evaluated in no study in Iran.

**Materials and Methods:** This study was conducted to compare differences in marital and sexual satisfaction in wives and husbands based on the 300 infertile wives and husbands (150 couples) in 3 Vali-e-Asr, Mirzakuchak Khan, and Shariati Reproductive Health Centers affiliated to Tehran University of Medical Sciences, Tehran, Iran, infertility Classification was determined through medical records. Four exposure categories were possible: female factor, male factor, mixed factor and unexplained factor infertility. Data was collected by three questionnaires including demographic characteristics; Marital Satisfaction Questionnaire (MSQ) and Sexual Satisfaction Questionnaire (SSQ) which were completed through interviews. Higher scores in questionnaires inspecting marital and sexual satisfaction indicates lower satisfaction.

**Results:** The outcomes, including differences in marital satisfaction and sexual satisfaction in wives and

husbands based on infertility diagnosis. In general, in infertile couples, wives had less marital and sexual satisfaction than their husbands. When a female factor was the cause of infertility, wives showed significantly less marital satisfaction than wives with other factors (male, mixed and idiopathic). Also, husbands with a female factor had less marital satisfaction than husbands with other factors. When a male factor was the cause of infertility, wives and husbands showed significantly less sexual satisfaction than wives and husbands with other factors ( $p<0.05$ ). Infertility diagnosis has a significant impact on infertile couple s in marital and sexual satisfaction.

**Conclusion:** Health care professionals can explain the gender differences and encourage them to share their feelings with each other, which may help couples to cope with the communication problems they may experience.

**Key words:** Infertility, Infertility diagnosis, Marital relationship, Marital satisfaction, Sexual satisfaction.

## P-24

### Results from a randomized, controlled trial study evaluating the effects of infertility

Vizheh M<sup>1</sup>, Pakgozar M<sup>2</sup>.

1. Faculty of Midwifery, Islamic Azad University, Branch of Mahabad, Mahabad, West Azerbaijan, Iran.
2. Department of Reproductive Health, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran.

Email: maryamvizheh@yahoo.com

**Introduction:** Infertility is a major cause of marital problems and sexual dissatisfaction. This study was conducted to determine the effects of counseling on infertile men's marital and sexual satisfaction.

**Materials and Methods:** This study was performed as a Randomized Controlled Trial (RCT) in which 100 infertile men who were visited at Reproductive Health Research Center Tehran, Iran, were randomly assigned into two groups: intervention (n=50 men) and control (n=50 men). Intervention was defined as three counseling sessions per week, each lasting 60-90 minutes. Counseling in the intervention group was conducted separately for each one. Demographic characteristics and marital and sexual satisfaction were investigated using three questionnaires through interviews. The outcomes, including changes in marital satisfaction and sexual satisfaction, were compared between the two groups three months later.

**Results:** Based on the data collected three months after the intervention period, the mean scores of marital satisfaction in intervention and control groups were  $45.48 \pm 9.55$  verses  $50.08 \pm 11.43$  ( $p=0.042$ ). Scores of sexual satisfaction in intervention and control groups were  $33.37 \pm 7.09$  verses  $36.63 \pm 6.52$  ( $p=0.025$ ), respectively. It should be noted that higher scores in questionnaires inspecting marital and sexual satisfaction indicates lower satisfaction.

**Conclusion:** Infertility counseling improves marital and sexual satisfaction in infertile men.

**Key words:** Infertility, Counseling, Marital relationship, Marital satisfaction, Sexual satisfaction.

## P-25

### Decreased expression levels of TNP1 and TNP2 genes in testis tissues of infertile men referred to Royan Institute

Heaydaryan N<sup>1,2</sup>, Eelaminejad Z<sup>1,2</sup>, Favaedi R<sup>2</sup>, Sadighi Gilani MA<sup>3</sup>, Shahhoseini M<sup>2</sup>.

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: m.shahhoseini@royaninstitute.org

**Introduction:** Many cases of male infertility associated with a severe impairment of spermatogenesis. During the last stage of spermatogenesis (spermiogenesis) the haploid sperm chromatin undergoes a drastic change at epigenetic level, in the way that, all of the histones are deposited first by transition proteins and then by protamines, the process causing to sperm DNA compactness. Sperm chromatin compaction is crucial to protect sperm DNA during transport through the female reproductive tract and fertilization. The transition nuclear proteins (TNP1 and TNP2) are encoded by TNP1 and TNP2 genes, and are the major chromatin basic proteins during the first steps of spermiogenesis.

**Materials and Methods:** This study aimed to determine the expression of TNP1 and TNP2 genes in testis tissue of infertile men, and verify a Local ethical approval was gained for this study and informed consent was given by patients. Testicular biopsies were collected from 20 infertile men referred to ROYAN institute and underwent testicular sperm extraction (TESE). Through pathological and spermogram analyses, these samples distributed into 4 groups: Hypospermatogenesis (positive control), Severe oligoasthenoteratozoospermia, complete maturation arrest at spermatid level and Sertoli cell only syndrome (negative control). Each group contained 5 samples. Using reverse transcription and quantitative real-time PCR reaction (qRT-PCR) methods, the expression profile of TNP1 and TNP2 genes were evaluated.

**Results:** The data significantly showed lower expression levels of TNP1 and TNP2 genes in testis tissues of all patient groups compared to positive control.

**Conclusion:** Our finding supported the previous studies considering the lower expression of TNP genes correlate with defective spermatogenesis.

**Key words:** Spermiogenesis, TNP, Male infertility.

## P-26

### Decreased expression levels of PRM1 and PRM2 genes in testes of infertile men referred to Royan Institute

Eelaminejad Z<sup>1, 2</sup>, Heaydaryan N<sup>1, 2</sup>, Favaedi R<sup>2</sup>, Sadighi Gilani MA<sup>3</sup>, Shahhoseini M<sup>2</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: m.shahhoseini@royaninstitute.org

**Introduction:** Spermatogenesis is a complex and unique process in male reproductive system which transforms diploid spermatogonia into differentiated haploid spermatozoa. The final stage of this process needs extensive chromatin condensation. This condensation occurs by replacement of histones by transition proteins which are subsequently replaced by protamines.

**Materials and Methods:** Protamines are the major nuclear sperm proteins that two types of them are known as protamine 1 (PRM1) and protamine 2 (PRM2). Several functional studies have been reported for these proteins through spermatogenesis, such as: condensation of the sperm nucleus, protection of the paternal genetic message delivered by the spermatozoa and involvement in the epigenetic imprinting of the paternal genome. In this study the expression profiles of PRM1 and PRM2 genes were evaluated in testis tissues of infertile men. Ethical approval and informed patient consent was gained for the use of tissue samples. Testicular biopsies were collected from 20 infertile men referred to Royan Institute and underwent testicular sperm extraction (TESE). Through pathological and spermogram analyses, these samples distributed into 4 groups: Hypospermatogenesis (positive control), Severe oligoasthenoteratozoospermia, complete maturation arrest at spermatid level and Sertoli cell only syndrome (negative control). Each group contained 5 samples.

**Results:** Total RNA was extracted from the tissue samples. After synthesis of first-strand cDNA, quantitative real-time PCR was performed using designed PRM1 and PRM2 primers. qRT-PCR analysis significantly revealed lower expression of PRM1, PRM2 in all groups compared to positive control.

**Conclusion:** Our finding supported a correlation between men infertility and protamine deficiency in infertile men referred to Royan Institute.

**Key words:** Spermatogenesis, PRM1, PRM2, Male infertility.

## P-27

### Effect of cadmium on oxidative stress of testes in adult male mice

Alaei S<sup>1</sup>, Monsefi M<sup>2</sup>.

1. Department of Biology and Anatomy, College of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.



2. Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran.  
Email: s.alaei@sbmu.ac.ir

**Introduction:** Cadmium ( $Cd^{+2}$ ) is a heavy metal and an environmental pollutant which is toxic to humans and animals. It is widely disturbed in the environment as a result of its diverse use in industries. Cadmium is transferred in food chain from water and soil to plants, animals and human. Human absorb cadmium by ingestion of contaminated foods and water, inhalation of polluted air or from smoking. According to WHO reports, approximately 2 to 6% of cadmium ingested, is absorbed into the body and causes oxidative stress. High concentration of oxidative stress is a powerful mechanism that can lead to sperm damage, deformity and eventually male infertility. Lipid peroxidation assay is used as an index of oxidative stress and is the organic expressions of oxidative stress in biological systems. Our previous studies showed that cadmium has adverse effects on spermatogenesis, sperm motility, count, maturation, testis structure and reproductive enzymes by accumulating in reproductive tissues and causes infertility in adult male mice. So, in this study we investigated the effect of Cadmium on oxidative stress in testes of adult male mice.

**Materials and Methods:** 30 Balb/c adult male mice divided to three groups (C, I and II) which was respectively received 0, 23 and 50 mg/kg of cadmium chloride in 0.5 ml distilled water for 45 days. At the end of the experiment, the mice were sacrificed under deep anesthesia and their testes were removed. Lipid peroxidation in testes was determined by measuring the amount of malondialdehyde (MDA) production.

**Results:** The formation of malondialdehyde, an indication of membrane lipid peroxidation, increased significantly in testes of group II compared with the control group.

**Conclusion:** According to these results and our previous studies we concluded that cadmium has adverse effects on male reproductive system. It causes oxidative stress which lead to infertility by destroying testes tissue and normal spermatogenesis.

**Key words:** Cadmium, Testis, Oxidative Stress, Infertility.

## P-28

### Adjuvant growth hormone therapy in antagonist protocol in poor responder

Eftekhar M<sup>1</sup>, Mohammadian F<sup>2</sup>, Aflatoonian A<sup>1</sup>.

1. Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Zanjan University of Medical Sciences, Yazd, Iran.

Email: mohamadian@zums.ac.ir

**Introduction:** The incidence of poor ovarian response in controlled ovarian stimulation (COH) has been reported in 9-24% of in vitro fertilization-embryo transfer (IVF-ET) cycles. Growth hormone augments the effect of gonadotropin on granulosa and theca cells,

and plays an essential role in ovarian function, including follicular development, estrogen synthesis and oocyte maturation. The aim of this study was to assess IVF-ET cycle outcome after the addition of growth hormone in antagonist protocol in.

**Materials and Methods:** Eighty-two poor responder patients selected for ART enrolled the study and were randomly divided into two groups. Group I (GH/HMG/GnRHant group, n=40) received growth hormone/gonadotropin/GnRH antagonist protocol and group II (HMG/GnRHant group, n=42) received gonadotropin/GnRH antagonist protocol.

**Results:** The number of retrieved oocytes was significantly higher in GH/HMG/GnRHant group than HMG/GnRHant group,  $6.10 \pm 2.90$  vs.  $4.80 \pm 2.40$  ( $p=0.035$ ) and the number of obtained embryos was also significantly higher in GH/HMG/GnRHant group than HMG/GnRHant group,  $3.7 \pm 2.89$  as compared to  $2.7 \pm 1.29$  ( $p=0.018$ ). There were no significant differences between groups regarding implantation, and chemical and clinical pregnancy rates.

**Conclusion:** Our study showed that co-treatment with growth hormone in antagonist protocol in patients with a history of poor response in previous IVF-ET cycles did not increase pregnancy rates.

**Key words:** Assisted reproductive technology, Poor Responder, Growth hormone, Antagonist protocol.

## P-29

### Transfer of blastocysts derived from frozen-thawed cleavage stage embryos improved ongoing pregnancy

Mohammadian F<sup>1</sup>, Eftekhar M<sup>2</sup>, Aflatoonian A<sup>2</sup>.

1. Department of Obstetrics and Gynecology, Zanjan University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: mohamadian@zums.ac.ir

**Introduction:** The fertilization rate in assisted reproductive technology (ART) cycles is about 70 %; however, only half of cleavage embryos advance towards blastocyst stage in day 3 and only one-third of good quality embryos will develop to blastocysts. It has been proposed that prolonged culture may lead to the development of embryos with higher implantation capacity. The aim of our study was to compare the transfer of embryos that are cryopreserved in cleavage stage after thawing with the transfer of embryos after thawing and culture in sequential media until blastocyst formation.

**Materials and Methods:** In this prospective clinical study, we have evaluated 134 cycles of ART treatment for infertility. Frozen embryos were thawed and then cultured in sequential media until blastocyst stage in blastocyst group and were compared with thawed embryos in cleavage stage group.

**Results:** Implantation rate was significantly higher in blastocyst group (30%) compared to cleavage group



(17%). No statistical differences were reported in chemical and clinical pregnancy rates between groups. Ongoing pregnancy rate was significantly higher in blastocyst group compared to cleavage group (42.9 vs 24.6%).

**Conclusion:** Our results indicated that blastocyst formation after thawing of cleavage stage embryos is a good predictor for embryo viability and pregnancy outcome.

**Key words:** Blastocyst, Cryopreserved embryo, Cleavage embryo, Pregnancy outcome.

### P-30

#### Calcium overload induced apoptosis in human normozoospermia and teratozoospermia

Ghani E<sup>1</sup>, Keshtgar S<sup>1</sup>, Habibagahi M<sup>2</sup>, Ghannadi A<sup>3</sup>, Kazeroni M<sup>3</sup>, Hosseini A<sup>2</sup>.

1. Department of Physiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Immunotherapy laboratory, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Shiraz Infertility Center, Shiraz, Iran.

Email: keshtgars@yahoo.com

**Introduction:** Teratozoospermia is a common cause of human infertility. The percent of live sperm in each specimen is a critical factor for determination of fertility rate. It has been shown that sustained intracellular calcium overload plays a major role in apoptosis. We investigated the biological significance of apoptosis in normozoospermia relative to teratozoospermia in the presence of calcium ionophore.

**Materials and Methods:** Teratozoospermic (n=8) and normozoospermic (n=10) semen samples were selected based on WHO criteria. Washed sperm incubated in sperm medium, DMSO, or calcium ionophore A23187 (10 µM) at 37°C for 1 h under 5% CO<sub>2</sub>. Apoptosis in all experimental groups were analyzed by flow cytometry using the Annexin V/propidium iodide (PI) binding assay.

**Results:** Control and DMSO groups of teratozoospermia had significant higher level of necrotic and dead cells compared to normozoospermia; however, the percent of early and late apoptosis did not show any significant differences. The percent of early and late apoptosis increased in normozoospermia and teratozoospermia after incubation with calcium ionophore. No significant differences were found in the level of necrotic cells between control and ionophore treated groups.

**Conclusion:** Our results suggest that human sperm are susceptible to apoptosis in the presence of sustained intracellular calcium overload; however, the percent of necrotic cells did not change. This revealed that apoptosis and necrosis in sperm have different inducers.

**Key words:** Apoptosis, Teratozoospermia, Normozoospermia, Calcium Ionophore.

### P-31

#### Effect of nano-zinc oxide on doxorubicin-induced oxidative stress and sperm disorders in adult male Wistar rats

Badkoobeh P<sup>1</sup>, Parivar K<sup>1</sup>, Kalantar SM<sup>2</sup>, Hosseini SD<sup>3</sup>, Salabat AR<sup>4</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Razi Vaccine and Serum Research Institute, Central Area Branch, Arak, Iran.
4. Department of Chemistry, Arak University, Arak, Iran.

Email: kazem\_parivar@yahoo.com

**Introduction:** Doxorubicin (DOX), an anthracycline antibiotic, is a widely used anticancer agent. In spite of its high antitumor efficacy, the use of DOX in clinical chemotherapy is limited due to diverse toxicities, including gonadotoxicity. So we investigated the protective effect of nano-zinc oxide (nZnO) as an established antioxidant on DOX-induced testicular disorders. So we investigated the protective effect of nano-zinc oxide (nZnO) as an established antioxidant on DOX-induced testicular.

**Materials and Methods:** In this experimental study 24 adult male Wistar rats were divided into four groups including one control and three experimentals (6 rats per group). They were been received saline (as control), DOX alone (6 mg/kg body weight, i.p.), nZnO alone (5 mg/kg body weight, i.p.), and nZnO followed by DOX. Animals were sacrificed 28 days after treatment and evaluations were made by sperm count and measuring sex hormone levels in plasma. Also total antioxidant power (TAP) and lipid peroxidation (LPO) in plasma were tested. Data was analyzed with SPSS-14 and one way ANOVA test. P-values <0.05 were considered to be statistically significant.

**Results:** In the DOX-exposed rats significant differences compared with the control group were found (p<0.05) in plasma total antioxidant power (TAP) (425.50±32.33 vs 493.33±18.54 mmol/mL), Lipid peroxidation (LPO) (3.70±0.44 vs 2.78±0.68 µmol/mL), plasma testosterone (3.38±0.69 vs 5.40±0.89 ng/dl), LH (0.26±0.05 vs 0.49±0.18 mIU/mL), sperm count (157.98±6.29 vs 171.71±4.42 10<sup>6</sup>/mL) and DNA damage (11.51±3.45 vs 6.04±2.83 %). Coadministration of nZnO significantly improved DOX-induced changes (p<0.05) in plasma TAP (471.83±14.51 mmol/mL), LPO (2.83±0.75 µmol/mL), plasma testosterone (5.00±1.07 ng/dl), LH (0.52±0.08 mIU/mL), sperm count (169.13±5.01 10<sup>6</sup>/mL) and DNA damage (7.00±1.67 %).

**Conclusion:** At the dose designed in the present investigation cytoprotective role of nano-zinc oxide through its antioxidant potential is illuminated in DOX-induced male gonadotoxicity.

**Key words:** Doxorubicin, Nano-zinc oxide, Antioxidant, DNA damage, Total antioxidant power, Lipid peroxidation, spermatogenesis.

### P-32

#### No association between the NAT2 803A/G and 857G/A polymorphisms and risk of endometriosis

Fayez D<sup>1</sup>, Irani Sh<sup>1</sup>, Saliminejad K<sup>2</sup>, Soltanghorae H<sup>2</sup>, Ahani A<sup>2</sup>, Kamali K<sup>2</sup>, Edalatkhah H<sup>2</sup>, Sharifi R<sup>2</sup>, Khorram

**Khorshid HR<sup>2</sup>.**

1. Department of Biology, Faculty of Sciences, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: H.Khorramkhorshid@avicenna.ac.ir

**Introduction:** Endometriosis is a heterogenous condition which affected by multiple susceptibility genes as well as environmental factors. Increasing number of studies show that the N-acetyltransferase 2 (NAT-2) gene polymorphisms are involved in development of endometriosis. The aim of this survey was to evaluate whether NAT2 gene polymorphisms are associated with susceptibility to endometriosis.

**Materials and Methods:** Totally, 141 women with diagnosis of endometriosis (Stage I-IV) and 157 healthy women who referred to Avicenna Infertility Clinic were included. DNA was extracted from peripheral blood using salting out method. The allele and genotype frequencies for the two polymorphisms, NAT2 803 A/G and 857 G/A, were determined using PCR-RFLP method. Statistical analysis was performed by X<sup>2</sup> test. A P-value < 0.05 was considered statistically significant.

**Results:** The genotype frequencies in the case and control groups of NAT2 803 A/G were (AA of 41.1% versus 41.1%, AG of 41.1% versus 46.2%, GG of 17.7% versus 12.7%, respectively) and in the case and control groups of NAT2 857 G/A were (GG of 51.8% versus 46.8%, GA of 44.7% versus 45.6%, AA of 3.5% versus 7.6%, respectively). No significant differences in the allele and genotype frequencies were found neither in the NAT2 803 A/G nor in the NAT2 857 G/A between the case and control groups.

**Conclusion:** Our results suggested that NAT2 803 A/G and 857 G/A polymorphisms were not associated with endometriosis in Iranian population.

**Key words:** Endometriosis, N-acetyltransferase 2, Polymorphism, PCR-RFLP.

**P-33**

**Vitamin D receptor FOKI polymorphism in women with recurrent abortions in Iranian population**

Sharifi R<sup>1,3</sup>, Shiravi A<sup>1</sup>, Zarei S<sup>2</sup>, Khorram Khorshid HR<sup>3</sup>, Ahani A<sup>3</sup>, Fayezi D<sup>3</sup>, Bahadori T<sup>4</sup>, Soltanghorae H<sup>3</sup>.

1. Damghan Azad University, Biology Faculty, Damghan, Iran.
2. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
4. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: soltan@avicenna.ac.ir

**Introduction:** Recurrent spontaneous abortions (RSA) are a frequent reproductive problem, which affecting 1 - 2% of women of reproductive age. There are many different causes of recurrent pregnancy loss. It seems be to an association between receptor vitamin D (VDR)

gene polymorphisms and the risk of recurrent spontaneous abortion (RSA) on the basis of published case-control studies for the VDR gene polymorphisms and similar disorders. Exploring the Relationship between the recurrent abortion and the polymorphism of vitamin D receptor's (VDR).

**Materials and Methods:** The case-control study included 126 women suffering from three or more consequent recurrent abortions referred to Avicenna Infertility clinic and 74 healthy controls with at least two live children. In this study DNA extracts of blood samples were used and PCR-RFLP applied for VDR gene (fok1) polymorphism detection. The Mann Withney Test is used to evaluate the results and p-value less than 0.05 was considered significant.

**Results:** The frequency of the fok1 (rs10735810 T/G) polymorphism genotypes in case group were as: normal (TT): 48.4%, heterozygote (TG): 39.7%, homozygote (GG):11.9% and in control group were (TT): 46.9%, (TG): 25.7%, (GG): 9.5%. The association between case and control groups was significant (p=0.037).

**Conclusion:** This study suggests this VDR polymorphism is a genetic determinant for the risk of recurrent abortion in Iranian women.

**Key words:** RSA, Polymorphism, VDR.

**P-34**

**Comparative effects of Cyclohexyl and Benzyl Ester administration on quantitative indices of rat seminiferous tubules**

Khanehzad M<sup>1</sup>, Noori Mugahi MH<sup>2</sup>, Kameli M<sup>2</sup>.

1. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Histology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Email: noorimoo@tums.ac.ir

**Introduction:** According to highlighted role of men in family planning, the use of reversible methods of contraception with no adverse effects on androgen-dependent metabolic reactions is very important. In order to achieve a conductor combination in the designing contraceptive drugs, in the study, the effect of two: Benzyl Ester and Cyclohexyl on histological parameters of seminiferous tubules was investigated.

**Materials and Methods:** This study was conducted on the 36 rat Sprague-Dawley with weighing 250-300 g and least 100 days. Then they were divided into two groups containing 13 mice and one group containing 10 mice and were Received respectively 15 mg/kg of Cyclohexyl and Benzyl Ester and Normal Saline as subcutaneous injection daily for 60 days. Next, samples of testicular fixative were stained by the usual method (H&E) and Histological parameters of seminiferous tubules were measured through Stereometry and Morphometry methods.

**Results:** Reduce the Perimeter of seminiferous tubules in the group of Benzyl Ester had significant difference with the two other groups (p<0.001), While the differences diameter of the seminiferous tubules in the

two groups was significant compared with the control group ( $p < 0.05$ ) and there was not noticeable difference among the three studied groups in terms of seminiferous tubules number.

**Conclusion:** Benzyl ester was more effective on Histological parameters of seminiferous tubules. Thus with eliminating the cardiovascular effects and Conservation the contraceptives properties, It can be used as a guide combination.

**Key words:** Benzyl Ester, Cyclohexyl, Morphometry, Seminiferous tubules, Stereometry.

### P-35

#### Investigation on the association of cytokine gene polymorphisms with recurrent miscarriage

Bahadori M<sup>1</sup>, Zarei O<sup>2</sup>, Zarei S<sup>3</sup>, Zarnani AH<sup>4</sup>, Jeddi-Tehrani M<sup>1</sup>.

1. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
2. Department of Pharmaceutical Biotechnology, Medical Sciences Faculty of Pharmacy, Tabriz University, Tabriz, Iran.
3. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
4. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: tannaz.bahadori@yahoo.com

**Introduction:** Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy losses prior to the 20th week of gestation. Inflammatory cytokine cascades have been implicated in the pathogenesis of RM. Research efforts have focused on studying single nucleotide polymorphisms (SNP) in candidate cytokine genes in RM women. The aim of this study was to survey the frequency of the interleukin IL-1 $\beta$  (-31 T/C, -511 C/T and +3954 C/T), IL-1RN (+9589 A/T, +8061 C/T and +11100 T/C), IL-10 (-592 A/C, -819 C/T and -1082 A/G) and IL-17 (-197 G/A) gene promoter polymorphisms in Iranian women with RM as compared to normal women.

**Materials and Methods:** In this case-control study, 105 RM women as the case and 75 healthy women with a history of two successful deliveries, without any pregnancy complications, as the control groups, were selected. Blood samples were recruited from Avicenna Infertility Clinic, Tehran, Iran. Polymerase Chain-Reaction and Restriction Fragment Length Polymorphism were performed to assess the frequency of the gene polymorphisms. The frequencies of the polymorphisms were calculated and compared between the case and the control groups.

**Results:** The data showed significant differences in IL10 promoter gene polymorphism (-819 C/T) frequencies between RM women and controls. However there were not any significant differences in the frequencies of interleukin IL-1 $\beta$ , IL-1RN, IL-10 (-592 A/C and -1082 A/G) and IL-17 polymorphisms between normal and RM women.

**Conclusion:** Our findings suggest IL-10 (-819 C/T)

polymorphism as a risk factor for RM.

**Key words:** Abortion, Polymorphism, Interleukin, Cytokine.

### P-36

#### Antioxidants supplementation in thawing extenders improve human sperm quality

Jafarbeik Irvani N<sup>1</sup>, Sadeghi MR<sup>2</sup>, Safarian Sh<sup>1</sup>, Lakpour N<sup>3</sup>.

1. Department of Biology, Faculty of Sciences, Tehran University, Tehran, Iran.
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: sadeghi@Avicenna.ac.ir

**Introduction:** Cryopreservation of human sperm is generally used to bank sperm for men undergoing radiation therapy, chemotherapy and ejaculatory dysfunction. Regrettably thought, cryopreservation has significant effect on frozen-thaw sperm parameters. The aim of present study was evaluation the effects of the reduced glutathione (GSH), vitamin E (E) and concomitant.

**Materials and Methods:** 20 pooled semen samples were collected. Each sample was frozen by using classical freezing method. All assays were done on fresh and frozen-thawed semen samples. In addition, the thawing medium was supplemented with (E), (GSH) and (E+GSH). Semen analysis was performed using CASA. Sperm DNA fragmentation was determined by SCSA and toluidine blue staining methods. Lipid peroxidation was determined by malondialdehyde assay. The occurrence of apoptosis was assessed using Annexin V/PI flow cytometry and also detection of the apoptotic genes expression using real-time RT-PCR.

**Results:** Flow cytometric results showed that the main cause of sperm death after freezing was necrosis although some sperms died based on apoptosis induction. Supplemented media can decrease apoptosis induction in the samples, although assessment of the typical apoptotic genes such as bax, bcl-2, caspase-3 and their ratios such as bax/bcl-2 indicated that our supplementation did not have any improving effects for apoptosis inhibition in comparison with the unsupplemented controls. In contrast, when we attended to the expression level of aif gene, especially for E supplementation, the inhibition of apoptosis could be explained based on the caspase independent pathway.

**Conclusion:** Based on the results supplementation of thawing medium with anti-oxidants such as E, G and E+G is partially able to control the rate of cell death possibly through inhibition of caspase independent pathway and prevention of oxidative stress damage on sperm plasma membrane and chromatin which finally cause partial increase in the sperm quality.

**Key words:** Semen freezing, Sperm, Vitamin E, Glutathione, Apoptosis.

### P-37

#### Association between a common polymorphism in the human aromatase gene and Iranian polycystic ovarian syndrome patients

Ghezelayagh Z<sup>1,2</sup>, Khosravifar M<sup>2</sup>, Ahmadi J<sup>3</sup>, Anisi Kh<sup>2</sup>, Sadegi M<sup>3</sup>, Akhlagi AA<sup>4</sup>, Shiva M<sup>3</sup>, Afsharian P<sup>2</sup>.

1. Department of Developmental Biology, University of Sciences and Culture, ACECR, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center of Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center of Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Department of Epidemiology and Reproductive Biomedicine, Reproductive Biomedicine Research Center of Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: pafshar@royaninstitute.org

**Introduction:** Polycystic ovarian syndrome is a common cause for infertility affecting 5-10% of women in reproductive age. Hyper-androgenemia is one of the main clinical features of this disorder. Aromatase is a member of Cytochrome P450 family and has a critical role in catalyzing the conversion of androgens to estrogens. It is responsible for keeping the homeostatic balance between estrogens and androgens so variations in its gene (CYP19A1) might be associated to syndromes of androgen excess such as PCOs. CYP19A1 is located on the long arm of chromosome 15. It is reported that several common polymorphisms such as SNP rs700519 (790C>T) on this gene are associated with serum androgen concentrations in PCO patients.

**Materials and Methods:** To investigate whether polymorphisms on Cyp19A1 are involved in PCOs disorder in Iranian population. A case control study including 100 individuals (45 control and 55 PCO patients) was performed to investigate the association of SNP rs700519 (Arg264Cys) with PCO patients. Polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) was implemented on the DNA isolated from blood samples to genotype this SNP.

**Results:** In this study CC and CT genotype were observed in both groups, but no homozygote TT was seen. The genotypic distributions of SNP rs700519 (CC, CT) of CYP19A1 gene in PCO patients (95.6%, 4.4%, respectively) were different from control groups (92.2%, 7.7%, respectively).

**Conclusion:** This study suggests that the common missense polymorphism rs700519 can be associated with PCOS; although a larger case control study must be performed.

**Key words:** Aromatase gene, CYP19A1, Polycystic ovarian syndrome, Polymorphism.

### P-38

#### Evaluation of expression pattern of the pluripotency factors of mice uterine tissue during different stages of the normal estrous cycle

Choobineh K<sup>1</sup>, Zavareh S<sup>1,2</sup>, Salehnia M<sup>3</sup>, Ghorbanian MT<sup>1,2</sup>, Paylakhi SH<sup>1</sup>.

1. School of Biology, Damghan University, Damghan, Iran.
2. Institute of Biology Sciences, Damghan University, Damghan, Iran.
3. Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Email: k.choobineh@gmail.com

**Introduction:** It was proposed many years ago that endometrial stem cells are responsible for the remarkable regenerative capacity of endometrium.

**Materials and Methods:** However, there is no report indicating changes of stem cells in different stages of the estrous cycle, therefore the aim of the 6-8 weeks old virgin female NMRI mice were considered as: proestrus, estrus, metestrus and di-estrus according to the cell types observed in the vaginal smear and were undergone hysterectomy operation. Immunohistochemical staining on cryostat sections of middle region of uterus horn and Quantitative real time PCR of complementary DNA of mRNA pluripotency markers (SOX-2, OCT-4, KLF-4, and NANOG) prepared from uterus tissue were performed.

**Results:** Immunofluorescence staining revealed expression of the pluripotency markers SOX-2, OCT-4, KLF-4, and NANOG in endometrium and myometrium, at all stages of estrous cycle, whereas, the locations of them were not different during estrous cycle. mRNA of Pluripotency markers were detected in all tested samples. Comparison of the normalized cycle of threshold (Ct) values revealed different levels of genes expression of Pluripotency markers. However, there were no significant differences among them at each stage of estrous cycle and during the estrous cycle.

**Conclusion:** This study showed expression of pluripotency markers at protein and mRNA levels in uterine tissue supporting the concept that endometrium contains a population of stem cells.

**Key words:** Pluripotency factors, Uterine tissue, Mice, Estrous cycle.

### P-39

#### Gene expression analysis of H2B histone variant hTSH2B in testis tissues of non-obstructive azoospermic patients referred to Royan Institute

Faghihi Zohani F<sup>1,2</sup>, Shahhoseini M<sup>2</sup>, Favaedi R<sup>2</sup>, Sadighi Gilani MA<sup>3</sup>, Shahzadeh Fazeli A<sup>1,2,4</sup>.

1. Department of Cellular and Molecular Biology, University of Sciences and Culture, Tehran, Iran.
2. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran.

Email: m.shahhoseini@royaninstitute.org

**Introduction:** 50% of infertility causes are because of male factors. Infertility in men usually is due to impaired spermatogenesis process. Human testis/sperm-specific histone H2B (hTSH2B) is a histone H2B variant specifically expressed in human testis tissues, from spermatogonia to mature sperm, which plays a crucial role during spermatogenesis and fertilization. The aim of this study is to investigate the expression levels of TSH2B mRNA in testis tissues of infertile men with non-obstructive azoospermia.

**Materials and Methods:** Samples were collected from infertile men referred to Royan Institute, who underwent testicular sperm extraction (TESE). Using quantitative real-time PCR (qRT-PCR) method, mRNA expression levels of TSH2B gene were compared in patients with non-obstructive azoospermia (n=15) in three groups (5 in each group) including: Sertoli Cell Only Syndrome (SCOS), complete maturation arrest at spermatid level, and hypo-spermatogenesis as positive control.

**Results:** Our data revealed lower expression levels of TSH2B in the two SCOS and maturation arrest patient groups rather than hypo-spermatogenesis.

**Conclusion:** Our data indicates that the lower levels of TSH2B gene expression can be associated with spermatogenic failure in non-obstructive azoospermia patients as a susceptibility factor for male infertility.

**Key words:** TSH2B, Male infertility, Spermatogenesis Impairment, TSH2B expression.

#### P-40

#### The effect of Omega3 supplementation on androgen profile and menstrual status in women

Vaziri N<sup>1</sup>, Nadjarzadeh A<sup>2</sup>, Daneshbodi H<sup>1</sup>, Dehghani Firouzabadi R<sup>3</sup>, Lotfi MH<sup>4</sup>, Mozaffari-Khosravi H<sup>2</sup>.

1. International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
4. Department of Biostatistics and Epidemiology, Faculty of Health, Shahid Sadoughi University, Yazd, Iran.

Email: niloofar\_vzr@yahoo.com

**Introduction:** There is evidence suggesting an association between androgen levels and menstrual status and probable beneficial effects of omega3 supplementation on PCOS. The aim of this study was to determine the effect of omega3 supplementation on sex hormone binding protein (SHBG).

**Materials and Methods:** This double-blind randomized clinical trial was conducted on 84 patients that suffer from PCOS. Subjects were randomly assigned to

consume either omega3 (3gr/day) or placebo for 8weeks. Data about weight, height and nutrient intake by 24hr recall as well as blood samples were collected before and after intervention. Serum concentrations of testosterone (nmol/L) and SHBG (nmol/L) was measured .FAI was also calculated as the ratio of testosterone to SHBG.

**Results:** Seventy eight patients completed the study. The mean of weight and BMI were 26.92±5.46 years and 31.69±4.84 Kg/m<sup>2</sup> respectively. There was no significant difference in mean age, weight, height, BMI and intake of energy and macronutrients between 2 study groups before and after treatment. All the patients entering the study had irregular periods. After the trial the percentage of regular menstruation in the omega3 group was more than the placebo group (p=0.049) (47.2% vs 22.9). Furthermore, testosterone concentration was significantly lower in the omega3 group compared with placebo, after supplementation (p=0.04). There was not any significant difference between two groups after intervention in cases of SHBG and FAI.

**Conclusion:** 8 weeks supplementation with 3 gr omega3 could reduce serum concentrations of testosterone. In addition, menstrual cycle becomes more regular in patients. But after 8weeks trial there was no statistical significant difference in the amount of FAI and concentrations of SHBG between two groups.

**Key words:** Polycystic Ovary Syndrome, Sex Hormone Binding Globulin, Free Androgen Index, Omega 3.

#### P-41

#### An exploratory study to introduce a practical ethical framework for reproductive health research

Farajkhoda T<sup>1</sup>, Latifnejad Roudsari R<sup>2</sup>, Abbasi M<sup>3</sup>.

1. Department of Midwifery, School of Nursing and Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Midwifery, School of Nursing and Midwifery, Patient Safety and Health Quality Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Shahid Beheshti Medical Law and Ethics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: farajkhoda\_t@yahoo.com

**Introduction:** Research in Reproductive Health (RH) has been located in the core of women's health research. Providing accurate information through conducting scientific and controlled research is essential, but increased number of research in the world especially in developing countries in RH area in order to introduce advanced technologies has been resulted in many unethical, illegal and abusive research on women, which needs particular attention to ethical issues by the practitioners who are involved in RH research. This study conducted to develop codes of ethics in research in RH in Iran.

**Materials and Methods:** The study was a sequential exploratory mixed method study, which was carried out in two phases between March 2010 and August 2012. In the first phase of the study through a modified three rounds Delphi study the professional code of ethics was developed. In the Round 1 Delphi, 45 Iranian academics and clinicians were purposively selected from four universities of medical sciences. Data were collected through sending a questionnaire including open-ended questions by E-mail and responses were analyzed using conventional content analysis. In the Round 2 Delphi, the draft of code of ethics developed in Round 1, delivered electronically to the participants who had taken part in the first Round. After data collection face and content validity (0.94) were calculated. The results of the Round 3 (Consensus percentage 94.98%) was accepted as professional code of ethics towards research on women SRH for RH providers.

**Results:** Emerged categories were 1) management of the research process 2) protection of participants' rights 3) third party consent 4) gender sensitive research and 5) conflict of interest.

**Conclusion:** This study has provided a practical ethical framework according to the socio-cultural context of Iran for all practitioners who are involved in research on women. Adherence to this framework may protect practitioners against unethical and illegal lawsuits and help them to respect their clients' RH rights.

**Key words:** Reproductive health, Research, Ethical framework.

#### P-42

##### Predictive value of follicular fluid Vitamin D in pregnancy rate of IVF program

Rahmani E<sup>1</sup>, Dehghani Firouzabadi R<sup>2</sup>, Rahsepar M<sup>2</sup>.

1. Department of Obstetrics and Gynecology, Bushehr University of Medical Sciences, Bushehr, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: rahmani@bpums.ac.ir

**Introduction:** Vitamin D is distinguished to be concerned in calcium-phosphate homeostasis and bone metabolism. Vitamin D is suggested critical roles in reproductive physiology.

**Materials and Methods:** This study was designed to investigate to find out 25OH-D levels in the follicular fluid (FF) and serum of infertile women. This prospective observational study included 221 infertile women participated in IVF cycle from 2010 to 2011. Serum and follicular fluid collected for vitamin D. Vitamin D deficient level, insufficient level, and sufficient level were defined as 10 ng/ml, 10-29 ng/ml and 30-100 ng/ml respectively. IVF cycle parameters and clinical pregnancy rate were compared with vitamin D level.

**Results:** In this study deficient level, insufficient level, sufficient level was 22.6%, 70.1% and 7.2%. No significant correlation was seen between pregnancy rate and serum vitamin D level ( $p=0.094$ ), follicular vitamin

D ( $p=0.170$ ). Serum and follicular fluid vitamin D level had significant correlation ( $p=0.000$ ).

**Conclusion:** Although vitamin D is one of the important hormones in the body, we did not find any correlation of serum and follicular vitamin D level with pregnancy rate in IVF cycle.

**Key words:** Vitamin D, In vitro fertilization, Follicular fluid, Pregnancy rate, 25OH-D.

#### P-43

##### Predicting endometriosis at laparoscopy in Iranian infertile woman: A case-control study

Jahanian Sadatmahalleh Sh<sup>1,2</sup>, Ashrafi M<sup>1</sup>, Akhoond MR<sup>3</sup>.

1. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Centre, Royan Institute for Reproductive Biomedicine, ACECR, Tehran-Iran.
2. Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
3. Department of Epidemiology and Reproductive Health at Reproductive Biomedicine Research Centre, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: shahideh.jahanian@modares.ac.ir

**Introduction:** Endometriosis affects woman's general physical and mental wellbeing. The most common presenting symptoms include dyspareunia, dysmenorrhea, pelvic pain, and infertility. This study aimed to determine the personal characteristic, demographic, reproductive factors, contraception and menstruation period associated with the presence of endometriosis. We also investigated the parameters that might predict the risk of endometriosis.

**Materials and Methods:** The subjects in the present retrospective study were women aged 16-46 years who underwent laparoscopy between 2010 and 2011 in Royan Institute. Of the 673 women were evaluated by laparoscopy, 341 patients had visual lesions of endometriosis (case group) while 332 subjects in the control group had no visual lesions of endometriosis. Statistical Analysis was done using Chi-square and t-test. Logistic regression was done to build a prediction model in endometriosis.

**Results:** In the logistic regression model, Gravidity ( $OR=0.815$ ,  $p<0.041$ ); family history of endometriosis ( $OR=2.765$ ,  $p<0.037$ ); history of galactorrhea ( $OR=1.838$ ,  $p<0.019$ ); history of pelvic surgery ( $OR=14.531$ ,  $p<0.001$ ); dysmenorrhea ( $OR=1.850$ ,  $p<0.006$ ); pelvic pain ( $OR=4.133$ ,  $p<0.001$ ); dyspareunia ( $OR=1.638$ ,  $p<0.017$ ); premenstrual spotting ( $OR=2.203$ ,  $p<0.002$ ); fatigue ( $OR=2.621$ ,  $p<0.006$ ) and diarrhea ( $OR=19.062$ ,  $p<0.005$ ) were significantly associated with endometriosis however, number of pregnancy ( $OR=0.815$ ,  $p<0.041$ ) was negatively related to endometriosis. The AUC value for the fitted logistic model was 0.798 (95% CI 0.761-0.831) showing a good predictive performance for the fitted logistic regression model.

**Conclusion:** Endometriosis is a considerable public health issues because of it affects many of women and associates with the significant morbidity. In this article we build a prediction model for endometriosis and show that it has good predictive performance. It can be used as a diagnostic tool in laparoscopy session to allow predicting the risk of endometriosis in infertile woman.

**Key words:** Endometriosis, Infertility, Laparoscopy.

#### P-44

#### A comparative study of luteal estradiol pre-treatment in GnRH antagonist protocols and in micro dose flare protocols for poor-responding patients

Rahsepar M<sup>1</sup>, Rahmani E<sup>2</sup>, Davar R<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Bushehr University of Medical Sciences, Bushehr, Iran.

**Email:** rahmani@bpums.ac.ir

**Introduction:** Poor response patients, following a standard ovarian stimulation protocol, have lower pregnancy rates compared with normal responders. This study aims to verify if luteal estradiol pre-treatment improves IVF/ICSI outcomes in a GnRH antagonist protocol as compared with a micro dose GnRH agonist protocol in poor-responding patients.

**Materials and Methods:** A total of 116 IVF/ICSI cycles were included in this prospective randomized single blind clinical trial. The selected women were randomly assigned to receive an estradiol pre-treatment in a GnRH antagonist protocol (daily oral Estradiol Valerate 4 mg preceding the IVF cycle from the 21st day until the first day of the next cycle) or in oral contraceptive pill micro dose GnRH agonist protocol.

**Results:** The patients in the luteal estradiol protocol required more days of stimulation ( $10.9 \pm 1.6$  vs.  $10.2 \pm 1.8$ ) and a greater gonadotropin requirement ( $3247.8 \pm 634.6$  vs.  $2994.8 \pm 611$  IU), yet similar numbers of oocytes were retrieved and fertilized. There was no significant difference between the two groups in terms of the implantation rates (9.8% vs. 7.9%), and the clinical pregnancy rates per transfer (16.3% vs. 15.6%).

**Conclusion:** This study demonstrates that the use of estradiol during a preceding luteal phase in a GnRH antagonist protocol can provide similar IVF outcomes when compared to a micro dose GnRH agonist protocol.

**Key words:** Poor responders, IVF outcome, Luteal phase, Estradiol, Micro dose protocol, GnRH antagonist.

#### P-45

#### Investigating the effects of Matricaria chamomile on prevention of abdominal adhesions among female rats

Fallahi S<sup>1</sup>, Najafian A<sup>1</sup>, Asadi R<sup>2</sup>, Dahghani Zahdani M<sup>3</sup>, Borzoi M<sup>1</sup>, Khakpour S<sup>1</sup>, Amjadi Golpaygani K<sup>2</sup>, Salimi M<sup>4</sup>, Esmail Shahrzad M<sup>4</sup>, Mahmodi F<sup>1</sup>, Hesam AA<sup>5</sup>, Dehghan A<sup>4</sup>.

1. Infertility and Reproductive Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
2. Hormozgan Veterinary Office, Bandar Abbas, Iran.
3. Department of Pathology, Shariati Hospital, Infertility and Reproductive Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
4. Student Research Committee, Hormozgan University of Medical Sciences (HUMS), Bandar Abbas, Iran.
5. Department of clinical psychology, Science and Research Branch, Islamic Azad University, Bandar Abbas, Iran.

**Email:** fallahi.leila@gmail.com

**Introduction:** Many abdominopelvic surgeries are performed everyday around the world due to appendicitis, cholecystitis, diagnostic laparotomy, excision of abdominopelvic masses, peritonitis, intestinal occlusions, trauma, visceral rupture and etc. Peritoneal adhesion is one of the most common complications of such surgeries. This complication poses huge costs to patients and society.  $\alpha$ -bisabolol that exists in chamomile can play a protective role to prevent these adhesions.

**Materials and Methods:** In this study, 50 female rats were divided into 5 groups: control group, normal saline, chamomile, chamomile plus L- Name, and chamomile plus indometacin. Intravenous ketamine was used to anesthetize the rats. After shaving and disinfecting the abdominal region, multiple scratches were made in the peritoneum and then, the abdomen was closed. After two weeks surgery, a relaparotomy was performed and a sample of the peritoneum was sent to the laboratory for adhesion grading and histopathological examination. The collected data was entered SPSS software for statistical analysis.

**Results:** The findings of this study showed that the rats of the chamomile group showed significantly less adherence, fibrosis, inflammation and vascular proliferation after surgery in comparison to other groups ( $p \leq 0.001$ ).

**Conclusion:** Despite many efforts made to prevent peritoneal adhesions with using different drugs, or various surgical techniques, no pleasant results have been achieved. Using herbal compounds such as chamomile is cheaper, has resulted in fewer complications and has an important effect in preventing abdominal adhesions.

**Key words:** Abdominal adhesions, Chamomile, Female Rat.

#### P-46

#### Expression of Toll-like receptors in human fallopian tubes

Amjadi FS<sup>1</sup>, Ghasemi N<sup>2</sup>, Salehi E<sup>1</sup>, Shakeri M<sup>3</sup>, Aflatoonian A<sup>2</sup>, Aflatoonian R<sup>4</sup>.

1. Department of Anatomy, Tehran University of Medical Sciences, Tehran, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Tehran Islamic Azad University of Medical Sciences, Tehran, Iran.

4. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: R.aflatoonian@gmail.com; abbas\_aflatoonian@yahoo.com

**Introduction:** The human female reproductive tract (FRT) is constantly deal with the invading pathogens. Recognition of these pathogens is attributed to the family of Toll like receptors (TLR) as a major part of the innate immune system. Reports from our laboratory and others have demonstrated the existence of TLRs 1-6 in the female reproductive tract. However, little has been done to identify TLRs 7-10 in the female reproductive tract, particularly in the fallopian tubes. To examine the expression of TLRs 7-10 in human fallopian tube tissue.

**Materials and Methods:** Using immunostaining techniques, distribution of TLR7-10 was studied in surgical sections from the uterine tubes, obtained from patients undergoing tubal ligation and hysterectomy for benign gynecological conditions. For genomic study, total RNA was extracted using TRI reagent standard protocol supplied by the manufacturer and treated with DNase I. First-strand cDNA synthesis was performed using oligo dT primers and the Superscript II reverse transcriptase system. RT-PCR was used to show the existence of TLR7-10 genes in fallopian tube epithelial tissue. TLR7-10 proteins were detected in the fallopian tube epithelium.

**Results:** TLR 7 and 9 were also observed weakly in stroma. TLR7-10 genes were expressed in human fallopian tube tissue.

**Conclusion:** This study indicates that TLR7-10 are expressed in fallopian tubes tissues, and may play an important role in microbial recognition, and in host defense against ascending infection.

**Key words:** Fallopian tube, Innate Immunity, TLRs, Reproductive Medicine.

## P-47

### Epigenetic mark alterations on Oct4 regulatory region through embryonal carcinoma cell differentiation

Favaedi R<sup>1</sup>, Baharvand H<sup>2</sup>, Shahhoseini M<sup>1</sup>.

1. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine Center, ACECR, Tehran, Iran.
2. Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

Email: shahhoseini@royaninstitute.org

**Introduction:** Embryonal carcinoma (EC) is a type of germ cell tumor that occurs in the ovaries and testes. The EC cells are the malignant counterparts of embryonic stem (ES) cells and provide a useful alternative to embryos for the study of mammalian cell differentiation. Transcription profile of both ES and EC cells undergoes remarkable changes onset of differentiation.

**Materials and Methods:** Pluripotency genes, expressed in undifferentiated state of maintained cells, are down regulated upon differentiation. One of these genes is POU domain homeobox gene of Oct4 which has a long up-stream regulatory region (2600 bp), consisting of proximal enhancer (PE), distal enhancer (DE) and proximal promoter (PP). This widespread regulatory region is target of many histone modifications as epigenetic marks which pronounced rapid changes by differentiation. The aim of this research was comparative evaluation of epigenetic profile of Oct4 regulatory region in embryonal carcinoma. In order to this aim a human EC cell line termed NT2, originated from testis tumor, grown under two different adherent and non-adherent culture conditions. Then differentiation induced to cells by retinoic acid (RA) treatment for 3 days. Regulatory regions of Oct4 gene studied by chromatin immunoprecipitation (ChIP) method coupled with real-time PCR technique to compare histone modifications on these regions as the epigenetic marks after differentiation.

**Results:** Results showed that by induced differentiation, the repressive epigenetic marks of hypoacetylation and methylation on lysine-9 of histone H3 occurred very effectively on the up-stream of Oct4, especially in PP region. Moreover comparison of the two culturing systems revealed that methylation of lysine-9 of H3 histone was more drastic in PE region of adherent cells rather than suspension cells. This epigenetic profile was in agreement with the difference observed in the expression level of Oct4 in these two culturing systems.

**Conclusion:** The current study clearly shows importance of epigenetic marks of gene regulatory regions especially proximal ones. Also, current findings showed the effective role of cell culture condition on the epigenetic regulation of gene expression.

**Key words:** Oct4, Epigenetic, Differentiation, Culture condition

## P-48

### Expression profile of MIF, CD74 and COX-2 in patients with endometriosis and normal endometrium in menstrual cycle

Mahdian S<sup>1,2</sup>, Shahhoseini M<sup>2</sup>, Noori E<sup>3</sup>, Salman Yazdi R<sup>4</sup>, Yaghmaei P<sup>1</sup>, Moini A<sup>3</sup>, Ramazanali F<sup>3</sup>, Afsharian P<sup>2</sup>, Aflatoonian R<sup>3</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: m.shahhoseini@royaninstitute.org; R.Aflatoonian@gmail.com

**Introduction:** MIF, Macrophage Migration Inhibitory



Factor, is a macrophage-secreted proinflammatory factor which is involved in T-cell activation, cell growth, apoptosis inhibition, and increases angiogenic factor production. MIF via its receptor, CD74, initiates a signaling cascade that leads to proliferation and survival of cells. Also, MIF binding to CD74 activates p38 signaling pathways that lead to positive effect on the expression of COX-2. The aim of this study was to evaluate the expression of MIF, CD74 and COX-2 genes in eutopic, ectopic and the control.

**Materials and Methods:** The level of MIF, CD74 and COX-2 genes expression was investigated by Q-PCR. Also, protein level of MIF in blood serum were measured by ELISA assay.

**Results:** It was shown that MIF, CD74 and COX2 expression in ectopic endometrium was higher than in eutopic and control endometrium. CD74 and COX2 expression in eutopic endometrium was higher than in control endometrium. However, there were some variations in mRNA expression of these genes in normal endometrium during menstrual cycle. Our results indicated various expressions in these genes in ectopic endometrium in proliferative and secretory phases, with a similar pattern with normal endometrium. Also it was shown that women with endometriosis had higher circulating levels of MIF protein as compared to normal controls.

**Conclusion:** Higher expression of MIF, CD74 and COX-2 genes in ectopic endometrium can be considered as a molecular biomarker for endometriosis development and pathophysiology. Variation in the expression of these genes in normal endometrium during menstrual cycle could play an essential role in reproduction, inflammation and endometrium reconstruction. Also, high level of MIF in blood serum in endometriosis could act as a biomarker in the diagnosis of endometriosis patients.

**Key words:** Endometriosis, MIF, CD74, COX-2.

## P-49

### Induction of human dental pulp stem cells differentiation into neural-like cells

Ebrahimi B<sup>1</sup>, Yaghoobi MM<sup>2</sup>, Mohammadi Kamal Abadi A<sup>3</sup>.

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2. Department of Biotechnology, Research Institute for Environmental Sciences, International Center for Science, High Technology and Environmental sciences, Kerman, Iran.

3. School of Dentistry, Kerman Medical University, Kerman, Iran.

Email: Ebrahimi.b@ssu.ac.ir

**Introduction:** Human dental pulp stem cells (DPSCs) have the neural crest origin and might have an intrinsic neurogenic potential. A number of studies have demonstrated the osteogenic, myogenic, chondrogenic, and odontogenic capacity of DPSCs. Different investigators have used basic fibroblast growth factor

(bFGF), retinoic acid and some commercial media such as Neurobasal A, B27 supplement for neural induction in DPSCs. In this study we investigated the effects of neural induction medium (NIM) optimized for neural induction of Bone Marrow Stem Cells developed by Woodbury *et al* 2000 on hDPSCs.

**Materials and Methods:** The cells were pre-induced by bFGF for 24 hours and then cultivated in NIM for one week. The composition of NIM was  $\alpha$ -MEM with 2% DMSO, 10ng/ml bFGF, 100 $\mu$ M butylated hydroxyanisole (BHA), 10 $\mu$ M Forskolin (Sigma), 25mM KCl, 2mM valproic acid and 5 $\mu$ g/ml insulin. Total RNA was extracted using the RNeasy kit (Qiagen) treated with DNase I and was reverse-transcribed. Real-time RT-PCR performed using specific primers for Nanog, cyclin D1, Nucleostemin, MAP2, GFAP and  $\beta$ -actin by FastStart SYBR Green Master ROX (Roche). The data were analyzed by LinRegPCR program. The Immunostaining was accomplished against GFAP and NF-H.

**Results:** Results showed that BHA and DMSO have toxic effects and therefore were omitted from the NIM. Following induction with modified NIM cell death decreased. The cells morphology changed into round cell bodies with sprouted processes. Some neurite-like processes were connected via synapse-like junctions between cells. The Q-PCR revealed down-regulation of nucleostemin (0.16) and cyclin D1 (0.7) expression. In addition, MAP2 and NF-H expression increased to 7.7 and 5.8 respectively after one week. The Immunostaining results confirmed GFAP and NF-H expression in differentiated cells.

**Conclusion:** Results demonstrated that modified NIM differentiates hDPSCs neutrally but not all cell population. It is possible that hDPSCs require a more potent inducer to overcome pluripotency regulatory networks, in contrast to bone marrow stromal stem cells (BMSCs) which respond quickly to NIM. Indeed, this neuro-inductive mixture could reveal a threshold for neural differentiation of DPSCs. Collectively hDPSCs could be a source of stem cells for future cell based therapies in neurodegenerative disorders.

**Key words:** Dental pulp stem cells, Neural differentiation, Real-time RT-PCR.

## P-50

### Cancer in pregnancy: A 10-year experience in Shahid Sadoughi Hospital, Yazd, Iran

Ghane Ezabadi M<sup>1</sup>, Karimi-Zarchi M<sup>2</sup>, Hekmatimoghaddam S<sup>3</sup>, Mortazavizade M<sup>4</sup>, Taghipour S<sup>5</sup>, Vahidfar M<sup>6</sup>, Vahedian H<sup>6</sup>, Forat M<sup>6</sup>, Shamsi F<sup>7</sup>.

1. Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2. Department of Obstetrics and Gynecology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

3. Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4. Department of Oncology, Shahvali Hospital, Yazd, Iran.

5. Department of Pathology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
6. Department of Internal Medicine, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
7. Department of Biostatistics and Epidemiology, School of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: drkarimi2001@yahoo.com

**Background:** Although occurrence of cancer during pregnancy is rare, it leads to high morbidity and mortality in both mother and fetus. Recent trends in prolongation of child-bearing age have made cancer-associated pregnancies more frequent than past. As yet there are few documents concerning cancer and its related treatment outcomes during pregnancy. This study aimed at describing clinical characteristics of pregnant women with cancer in the Shahid Sadoughi hospital in Yazd, Iran.

**Materials and Methods:** Case series were reviewed retrospectively, which included 19 pregnant women diagnosed with cancer in Shahid Sadoughi hospital from 2002-2012. Data collected comprised demographics, pregnancy characteristics and outcomes, type of cancer, clinical stage, treatment and oncological outcome.

**Results:** From 17 pregnant women with cancer, 4 women had gynecologic cancers and 13 had non-gynecologic cancers. The Following tumors were observed: breast (6) acute myeloblastic leukemia (3), uterine cervix carcinoma (3), ovary (1), chronic myelogenous leukemia (1), lymphoma (1), papillary carcinoma of thyroid (1), and pseudopapillary carcinoma of pancreas (1). The mean age of patients was 30.6 years, and the mean gestational age at diagnosis was 21.1 weeks. Surgical treatment was performed in 3 patients, 6 patients were treated by chemotherapy, and in 3 by both.

**Conclusion:** Although cancer during pregnancy is uncommon, it is considered an important problem due to unsuitable maternal and fetal outcomes and lack of standard management guidelines.

**Key words:** Cancer, Pregnancy, Outcome, Yazd.

## P-51

### The role of oxidative stress in diazinon-induced tissuestoxicity in Wistar and Norway rats

Salehi M<sup>1</sup>, Jafari M<sup>1</sup>, Kazemi M<sup>2</sup>.

1. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Email: maryam.salehi60@gmail.com

**Introduction:** Diazinon (DZN) is an organophosphate pesticide widely used in agricultural to control insects and in veterinary medicine to control ectoparasites. This study investigated the induction of oxidative stress in the brain, heart, and spleen of Wistar and Norway rats treated with acute doses of DZN.

**Materials and Methods:** Female Wistar and Norway

rats were treated with 25, 50, 100, and 200 mg/kg of DZN by intraperitoneal injection. The animals were sacrificed 24 h after treatment, and tissues were isolated and analyzed. The result of this study shows that DZN at higher doses increased the level of malondialdehyde, superoxide dismutase and glutathione S-transferase activities and decreased glutathione (GSH) level, lactate dehydrogenase, and cholinesterase activities in the brain, heart, and spleen of both rat strains.

**Results:** At these concentrations, DZN toxicity also lead to a significant decrease in catalase (CAT) activity in all tissues of Wistar rat and brain of Norway rat, while it increased heart CAT activity in Norway rat. However, the alteration of these parameters was observed at lower doses of DZN in Wistar rat.

**Conclusion:** These results suggest that DZN at higher doses induces the production of free radicals and oxidative stress in rat tissues and strains by alteration of antioxidant enzyme activity, depletion of GSH, and increasing lipid peroxidation. Induction of oxidative stress in DZN-treated rats is in the order of brain >heart >spleen. Wistar rats appear to be more sensitive to the effects of DZN on oxidative stress induction compared to Norway rat.

**Key words:** Diazinon, Antioxidant enzymes, Lipid peroxidation, Wistar and Norway rats.

## P-52

### Comparison of contraceptives in Iranian traditional medicine and modern medicine

Nekoolaltak M<sup>1, 2</sup>, Tansaz M<sup>2</sup>, Bioos S<sup>3</sup>, Mokaberinejad R<sup>3</sup>.

1. Faculty of Reproductive and sexual health, Nursing and Midwifery School, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Faculty of Iranian Traditional Medicine, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Faculty of Iranian Traditional Medicine, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Email: tansaz\_mojgan@yahoo.com

**Introduction:** Unintended pregnancy is a global problem. Worldwide of the approximately 210 million pregnancies occurring each year, 38% are estimated to be unintended and 22% end in abortion. Un-intended pregnancy is associated with substantial costs to health and social services, and emotional distress to women, their families and society as a whole. Also unintended pregnancy has negative health risk factors for mothers and children. Today there are many different contraceptives, but 217 million women have unmet needs for contraceptives yet. Many couples want to prevent or postpone the birth of next child but they do not access to contraceptives, or don't use contraceptives because of cultural and social obstacles or because of their side effects. Therefore searching and finding new methods for birth control is a per-manent necessity. It is expected oncoming methods will be more compatible with culture, test, physical and medical characteristics and life style of couples.

**Materials and Methods:** This study is a descriptive review on contraceptives in some of traditional Iranian medicine books to compare with modern To do this, we carefully scanned the main texts of Iranian traditional medicine such as Al-Qanun Fi Al-Tibb, (The canon of medicine) Ibn Sina (Bukhara 980-Hamadan, moalejat Aghili, and so. contraception related subjects under the entry of "gynecology", Contextual analysis was done and their discrepancies and congruities were summarized.

**Results:** This data categorized and compared with contraceptive in modern medicine. Contraception issue in traditional Iranian medicine can be divided to 9 parts: male contraceptives (local methods) 1. Mail contraceptives (local method) 2. Female contraceptives (local and systemic methods). 3. Emergency contraceptives. 4. Drugs for sterilization. 5. Contraceptives with effect in definite period. 6. Inhaling steam for vagina. 7. Contraceptives special for menstruations time. 8. Emotional and physical and positional aspects in sex. 9. Food dietary before sex for reducing fertility.

**Conclusion:** This study indicates there were many different drugs and substances with many usage methods for prevent pregnancy in traditional Iranian medicine, most of them were inexpensive and accessible. On the other hand, current contraceptives can't satisfy all of applicant families and many researchers are necessary for making newer, easier, and more acceptable with less side effect contraceptives. New clues for producing new contraceptives or promotion of current contraceptives can be achieved by reviewing, researching and clinical trials on traditional contraceptives. In this way Hu-man generation will be healthier, happier and more efficient by developing programmed pregnancies.

**Key words:** Unintended pregnancy, Contraceptives, Iranian traditional medicine.

## P-53

### Morphine effects on folliculogenesis in culture medium and parthenogenesis

Behzadi M<sup>1</sup>, Arjmand Sh<sup>2</sup>, Amini S<sup>3</sup>, Emami Meybodi MA<sup>3</sup>, Nematollahi-mmahani SN<sup>3</sup>.

1. School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
2. School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Anatomy, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

**Email:** Mina.behzadi11@gmail.com

**Introduction:** Morphine is a naturally occurring substance in the opium poppy, which acts as a powerful analgesic medication in order to relieve pain. Although the effects of morphine on ovarian physiology have been demonstrated to some extent, its effects on folliculogenesis remains poorly understood. The aim of this study was to assess the effects of morphine on

folliculogenesis in vitro and fertilization competency by follicle culturing.

**Materials and Methods:** First, sixteen young female mice were divided into treatment and control groups. The mice were addicted by oral morphine consumption for 21 days. The mice were then superovulated; the ovaries were removed and placed in PBS containing BSA. Cumulus oophorus complexes (COC) were mechanically released into a prewarmed buffer and evaluated for maturation status. Morphologically normal COCs were cultured for 18 hours in the presence of gonadotrophins for further maturation. MII oocytes were transferred to fertilization medium (HTF-BSA) and inseminated with swam up spermatozoa from cauda epididymis of NMRI mice with proven fertility. After 36 hours the fertilization rate was assessed by the development of MII oocytes to 2-cell stage embryos. The proportion of MII oocytes and 2-cell embryos in the different groups was compared by  $\chi^2$  test.

**Results:** The rate of maturation to MII oocytes in the follicles obtained from morphine dependent mice was comparable with the control group. Also, the fertilization rate and development to 2-cell embryos in morphine dependent animals was not statistically different from the control group.

**Conclusion:** According to this experiment we conclude that long term morphine administration followed by in vitro maturation and fertilization may sustain fertility in morphine dependent mice.

**Key words:** Morphine, In vitro fertilization, Parthenogenesis, Mice.

## P-54

### TLR 2, 3 and 4 expression and localization in the patients with the history of endometriosis

Aflatoonian R<sup>1</sup>, Mohebbi A<sup>1</sup>, Janan A<sup>2</sup>, Noori E<sup>1, 2</sup>, Nasri S<sup>1</sup>.

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine (ACECR), Tehran, Iran.
2. Department of Biology, Payam Noor University, Tehran, Iran.

**Email:** R.Aflatoonian@gmail.com

**Introduction:** Endometriosis is a benign disorder which is characterized by the presence of endometrial glandular and stromal cells in areas outside of the uterine. There are relationships between the female immune system and the occurrence of endometriosis. Toll-like receptors (TLRs) are a major family of PRRs which recognize specific pathogen associated molecular patterns (PAMPS). TLR2 is essential in the recognition of microbial lipopeptides and peptidoglycan derived from Gram-positive bacteria. TLR3 is a transmembrane receptor located on endocytic vesicles which binds double-stranded RNA (dsRNA), a component of some viruses. TLR4 is an essential receptor for recognition of LPS Gram Negative bacteria. The objective of this study is to clarify the expression of TLR 2, 3, 4 in the

ectopic and eutopic endometrium of women.

**Materials and Methods:** Normal and eutopic endometrium obtained with pipelle from endometrium of women without and with endometriosis, respectively. Ectopic samples obtained with laparoscopic procedure from patients with endometriosis. RT-PCR and Quantitative PCR was performed using the prepared cDNA and primers for TLR2, 3 and 4. Relative TLRs expression quantities were compared between three groups. Differences in normalized expression values between samples were tested for significance using ANOVA statistical test.

**Results:** RT-PCR and Q-PCR has been shown the significantly different expression of TLR2, 3 and 4 genes in all samples of the eutopic, ectopic and control groups.

**Conclusion:** Endometriosis is a very complex disease with a great impact on many women's quality of life. This disease affects roughly one in ten women of reproductive age. There are obvious associations between endometriosis and the immune system, and future strategies to treat endometriosis might be based on immunological concepts and methods. The different expression of TLR2, 3 & 4 in these three types of endometrial tissues may be a strong evidence of critical role of innate immune system in outbreak of endometriosis.

**Key words:** Endometriosis, Ectopic endometrium, Eutopic endometrium, Innate immune system, TLR.

## P-55

### Association between HLA-G genotypes and repeated implantation failure in Iranian couples

Kuroshli Z<sup>1,2</sup>, Gourabi H<sup>2</sup>, Bazrgar M<sup>2</sup>, Sanati MH<sup>2,3</sup>.

1.Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.

2.Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

3.Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

Email: gourabi@royaninstitute.org

**Introduction:** During pregnancy, the proper function of Immunogenetic and immunological factors is many important and disruption in these may often lead to reproductive failure. The HLA-G, as a tolerogenic molecule, expressed in cytotrophoblast cells and play an important role in the suppression of the immune response in maternal decidua. Today Interaction of the extracellular domains of the HLA-G protein with cell receptors of the immune system, including CD8, LILRB1, LILRB2 and the killer cell immunoglobulin-like receptor KIR2DL4 was well known.

**Materials and Methods:** We studied about association between HLA-G gene polymorphisms and repeated implantation failure (RIF). we used polymerase chain reaction (PCR) followed by sequencing technique for exon 2, 3, 4 and intron 2 of HLA-G gene in 100 couples

with two or more failed assisted reproductive technology (ART) in their history and 50 couples with normal fertility and had one or more child from same partner that referred to Royan Institute of Tehran.

**Results:** The obtained results indicate that some alleles of HLA-G gene including 0106, 010106, 01010106 and 0105N (null) alleles were significantly higher in the patient group compared to control group ( $p < 0.05$ ). So we suspect that nucleotide changes in coding regions of HLA-G gene (specially exon 3 and 4) can be effective on HLA-G function and so may have a role in changing the quality of implantation. On the other hand, nucleotide variation in intronic regions of HLA-G gene maybe have association with RIF.

**Conclusion:** The significant genotype-specific risk in failed ART group suggested that allelic variation, especially in exon3, exon4 and also in intron 2 of HLA-G gene can led to implantation failure in human embryos.

**Key words:** HLA-G, Implantation, ART, RIF

## P-56

### RG108 as a DNA methyltransferase1 inhibitor cannot improve developmental rate of B6D2F1

Zarei M, Dalman A, Shamaghdari B, Hadi M, Eftekhari-Yazdi P.

Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: peyazdi@yahoo.com

**Introduction:** In somatic cell nuclear transfer (SCNT), transferred nuclei is epigenetically essential to be reprogrammed from the somatic to the embryonic type, which is important for development of the cloned embryos. Since the DNA methylation is an important factor during embryonic preimplantation development; and also cloned embryos are hypermethylated with incomplete reprogramming of donor nucleus; in this study, we focused on the role of the RG108 as a DNA methyltransferase1 inhibitor in success rate of cloned preimplantation mouse embryos development and discuss whether RG108 can promote cloning efficiency?

**Materials and Methods:** SCNT cloned 2-cell stage B6D2 mouse embryos were treated with 10  $\mu$ M RG108 and embryo developmental rate was measured up to morula stage.

**Results:** Developmental rate to morula/blastocyst in RG108 treated were significantly lower than untreated cloned embryos (8% vs 33%, respectively).

**Conclusion:** The finding of the present study show that treated cloned embryos began to cleave and develop to the 4-cell stage with the time equivalent the fertilized embryos, but arrested in this stage. Therefore RG108 as a small molecule probably could not increase the developmental rate of treated cloned mouse embryos to morula/blastocyst stage.

**Key words:** Cloning, SCNT, DNA methylation, RG108.

### P-57

#### The correlation between follicular fluid pregnancy-associated plasma protein A levels, fertilization, and embryo quality in GnRH agonist and GnRH antagonist protocols in ART cycles

Mohammadian F<sup>1</sup>, Dehghani Firouzabadi R<sup>2</sup>, Mashayekhi M<sup>3</sup>, Eftekhari M<sup>2</sup>.

1. Department of Obstetrics and Gynecology, Zanjan University of Medical Sciences, Zanjan, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Department of Obstetrics and Gynecology, Arak University of Medical Sciences, Arak, Iran.

Email: mohammadian\_famaz@yahoo.com

**Introduction:** Determination of oocyte fertilization and embryo quality are one of the most important purposes in ART cycles. Follicular fluid provides an important microenvironment for development of oocytes and some biochemical characteristics of the follicular fluid, such as pregnancy-associated plasma protein-A (PAPP-A), may play an important role in prediction of success rate of ART. This study was performed to evaluate whether there was any difference in follicular fluid PAPP-A, fertilization, and embryo quality between GnRH agonist long protocol and flexible GnRH antagonist multiple-dose protocol in ART cycles.

**Materials and Methods:** A total of 100 women who were candidates for ART were enrolled the study and were divided into two groups, GnRH agonist (GnRHa) long protocol (n=51) and flexible GnRH antagonist (GnRHant) multiple-dose protocol (n=49). Follicular fluid sample was obtained from a single mature follicle and follicular fluid PAPP-A level; fertilization and embryo quality of the same oocyte were evaluated in both groups.

**Results:** There was no significant difference in the mean levels of follicular fluid PAPP-A between the GnRHa protocol and GnRHant protocol ( $3.5 \pm 1.4$  vs.  $3.8 \pm 1.9$ , respectively). The mean levels of follicular fluid PAPP-A in fertilized oocyte and good quality embryo were comparable in GnRHa and GnRHant protocols.

**Conclusion:** Our data indicated that no differences of follicular fluid PAPP-A levels were observed between cycles using GnRHa long protocol and those of using flexible GnRHant multiple-dose protocol.

**Key words:** Pregnancy-associated plasma protein A, Follicular fluid, GnRH agonist, GnRH antagonist, Assisted reproductive.

### P-58

#### Transplantation of insulin secreting cells derived from human umbilical cord mesenchymal cells into diabetic rats

Yari N<sup>1</sup>, Nematollahi-mahani SN<sup>2</sup>, Nabipour F<sup>3</sup>, Bazrafshani MR<sup>4</sup>.

1. Research and Clinical Center for Infertility, Shahid

Sadoughi University of Medical Sciences, Yazd, Iran.

2. Department of Anatomy, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Pathology, Kerman University of Medical Sciences, Kerman, Iran.
4. Department of Genetics, Kerman University of Medical Sciences, Kerman, Iran.

Email: nahidyari@yahoo.com

**Introduction:** Diabetes is the most common metabolic disorder in the world. Only less than half of the patients with common methods have optimal control of blood sugar. Cell therapy for treatment of diabetes have shown some advantages. However, lack of appropriate number of cells for transplantation and low rate of insulin secretion by differentiated cells have limited the utilization of adult stem cells in treatment of diabetes. The aim of the present study was to evaluate the potential of human umbilical cord matrix-derived mesenchymal (hUCM) cells to differentiate into insulin producing cells and to treat diabetic rats.

**Materials and Methods:** Transplantation of insulin secreting cells derived from human umbilical cord mesenchymal cells into diabetic rats for diabetes To differentiate into insulin-secreting cells, hUCM cells were cultured in the NCM and SCM. Expression of Pdx1 and Insulin was analyzed by RT-PCR. Different concentrations of glucose, was added to the culture medium to measure insulin and C-peptide secretion in differentiated cells. Immunocytochemistry evaluation was performed to confirm differentiation of hUCMs to insulin producing cells. Island-like cluster of cells were transplanted under the kidney capsule of diabetic rat.

**Results:** RT-PCR showed expression of Pdx1 and Insulin in differentiated cells. In response to different concentrations of glucose, the differentiated cells secreted insulin and C-peptide and insulin was detected by immunocytochemistry in these cells. Diabetic animals that received differentiated cells showed a significant reduction in blood sugar after 3 weeks and a balanced weight gain after 4 weeks.

**Conclusion:** The human umbilical cord matrix-derived mesenchymal cells could successfully differentiate into insulin producing cells in vitro. Also, transplantation of differentiated cells into diabetic rats could decrease blood sugar and improve weight gain.

**Key words:** Human umbilical cord derived mesenchymal cells, Diabetes, Differentiation into insulin-secreting cells.

### P-59

#### The polymorphism of MIF associated with endometriosis

Chekini Z<sup>1, 2, 3</sup>, Ramazanali F<sup>2</sup>, Irani Sh<sup>1</sup>, Afsharian P<sup>3</sup>, Aflatoonian R<sup>2</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Genetics, Reproductive Biomedicine

Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: pafshar@royaninstitute.org; r.afatoonian@gmail.com

**Introduction:** Macrophage migration inhibitory factor (MIF) is a key pro-inflammatory cytokine that is secreted by active macrophages accumulated in ectopic tissue of endometriosis. MIF was shown to promote angiogenesis by inducing endothelial cell proliferation or stimulating of VEGF expression. It plays an essential role in cell proliferation by negative regulation of p53 and stimulates the synthesis of PGE<sub>2</sub>, so finally triggers local estradiol synthesis in endometriotic stromal cells. MIF upregulates the expression of TLR4 in macrophages which cause over-transcription of cytokines and adhesion molecules through NF-KB pathway. There is a functional single nucleotide polymorphism/SNP (rs755622) in MIF promoter at position -173 (C/G) which is related to modified promoter activity. The aim of this study was to evaluate a functional MIF polymorphism is associated with endometriosis in Iranian population.

**Materials and Methods:** Genomic DNA of 30 patients with endometriosis and 40 unrelated controls were amplified via polymerase chain reaction (PCR). Restriction fragment length polymorphism (RFLP) was determined for -173G/C SNP in MIF promoter.

**Results:** Polymorphism of -173 G/C of the MIF was identified in control & patient groups. The distribution frequencies of G allele and C allele of -173 locus in MIF gene were 16% and 84%, respectively in endometriosis group, and they were 14% and 86%, respectively in control group.

**Conclusion:** The present study established G/C polymorphism in both group but no significant association was identified between control and case groups. Our group has detected over expression of MIF in same samples, therefore it seems that polymorphism of MIF can be allied with endometriosis. Although it needs to be confirmed by elevating the number of studied endometriosis patients.

**Key words:** Endometriosis, Macrophage migration inhibitory factor, Polymorphism.

## P-60

### Expression of vascular endothelial growth factor and its receptors in endometriosis

Noori E<sup>1,2</sup>, Nasri S<sup>1</sup>, Mahdian S<sup>2,3</sup>, Janan A<sup>2</sup>, Aghajani S<sup>2</sup>, Moini A<sup>2</sup>, Ramazani F<sup>2</sup>, Lakpour MR<sup>2</sup>, Afatoonian R<sup>2</sup>.

1. Department of Biology, Payam Noor University, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.

Email: r.afatoonian@gmail.com

**Introduction:** Endometriosis is a gynecological disease that affects women of reproductive age. This disease is defined by the growth of endometrium-like tissue outside the uterine cavity. Several factors have been founded that play an important role in the pathogenesis of endometriosis. The most important of them is vascular endothelial growth factor (VEGF). VEGF is a homodimeric glycoprotein which act as angiogenic agent by interaction with two tyrosine kinase receptors, Flt-1 (Fms-like tyrosine kinase-1 or VEGFR-1) and Flk-1/KDR (fetal liver kinase/kinase-insert domain receptor or VEGFR-2). The aim of this study was to compare the expression of VEGF and its receptors in women with and without endometriosis.

**Materials and Methods:** This study contain 3 groups (n=10). In patient with endometriosis, ectopic biopsies (ovarian endometrioma) and eutopic biopsies were obtained by laparoscopic procedure and pipelle respectively. In women with no sign of endometriosis, control biopsies gained with pipelle. The samples in each group were obtained in different stages of the menstrual cycle. Gene expression of VEGF was determined by RT-PCR and the quantitative level of gene expression was tested by Real Time PCR.

**Results:** VEGF gene and its receptors were expressed in all groups. However, Q-PCR analysis showed that Expression of these genes were variable in different phases of menstrual cycle and different groups. In eutopic endometrium of patients affected endometriosis, expression of VEGF, VEGFR1 and VEGFR2 were higher in compare to control and ectopic groups.

**Conclusion:** VEGF is an angiogenic factor involved in physiology and pathology of angiogenesis. Expression of VEGF and its receptors increase in eutopic endometrium of women with endometriosis but in ectopic lesions level of expression likely depends on lesion location, as we showed in ovarian endometrioma. Our data showed that ovarian endometriomas are lesions with Low angiogenic activity and low ability of remodeling the surrounding tissue.

**Key words:** Endometriosis, Angiogenesis, VEGF, VEGF Receptors

## P-61

### Prenatal stress induces metabolic impairment in adolescent male Wistar rat

Rostamkhani F<sup>1</sup>, Zardooz H<sup>2,3</sup>, Shirvani H<sup>4</sup>.

1. Department of Biology, Faculty of Sciences, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.
2. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Department of Physical Education, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran.

Email: shirinroostamkhani@yahoo.com

**Introduction:** A large number of studies have reported associations between prenatal stress and offspring lifetime consequences. Chronic gestational stress alters

maternal glucocorticoids and subsequently disturbs intrauterine environment which may lead to metabolic disorders in the offspring. The aim of this study was to investigate the effects of chronic prenatal stress on the metabolic parameters in adolescent male Wistar rat.

**Materials and methods:** We examined the effects of maternal 8 and 20 days foot-shock stress on body weight, plasma corticosterone, insulin, glucose, triglyceride and cholesterol concentrations of dams and offspring. Stress was induced by a foot-shock box twice a day (1 h/session) for 8 consecutive days beginning on E8 in 8-day stressed group and for 20 consecutive days beginning on E1 in 20-day stressed group.

**Results:** The results obtained from this investigation indicate that gestational chronic foot-shock stress arises maternal plasma corticosterone concentration. In addition, maternal plasma triglyceride and cholesterol concentrations significantly elevated following 20-day gestational stress. Prenatal stress induces lower birth weight and body weight gain in offspring. Furthermore, prenatal stressed offspring had significant elevation in plasma glucose concentration without marked alteration in plasma insulin, corticosterone, triglyceride and cholesterol concentrations.

**Conclusion:** These data suggest that prenatal stress could result in impaired glucose metabolism in the adolescent rats which is independent of timing of the stress exposure.

**Key words:** Prenatal stress, Corticosterone, Insulin, Glucose, Triglyceride, Cholesterol.

#### P-62

##### Study the of polymorphism of STK11 gene in polycystic ovary syndrome

Tarahomi N<sup>1, 2</sup>, Modaresi-Nejad M<sup>1, 2</sup>, Khosravifar M<sup>2</sup>, Shiva M<sup>3</sup>, Parvaneh A<sup>2</sup>.

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Endocrinology and Reproductive health at Reproductive Biomedicine Research Center, Royan institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: pafshar@royaninstitute.org

**Introduction:** Polycystic Ovary Syndrome is a common endocrinopathy affects 5-10% of women in reproductive age. Metformin is one of the most important drugs that used in women with Polycystic Ovary Syndrome under treatment of ovulation induction and STK11 gene is necessary for action of metformin. STK11 is a serine threonine kinase gene expressed in liver. It acts as a tumor suppressor and a metabolic enzyme which is the regulator of energy homeostasis and cell polarity. STK11 phosphorylates and activates adenosine monophosphate- activated protein kinase (AMPK). The aim of our study was to study of 2 single nucleotide polymorphisms.

**Materials and Methods:** Blood samples were obtained from infertile PCO women referred to Royan Institute and control groups (40 in each group). PCR- RFLP analysis was used to find variations of STK11 gene.

**Results:** T allele in exon 6 was observed in 25% and 18% of patient and fertile women, respectively. The single nucleotide polymorphism (rs121913325) was not detected in exon 8.

**Conclusion:** We detected LOH (loss of heterozygosity) in exon 6 significantly different in both groups, which it showed this SNP can be related to PCO failure treatment. Data showed rs121913325 SNP in exon 8 was not associated with PCOS.

**Key words:** Polycystic ovary syndrome, STK11, Restriction Fragment length polymorphism.

#### P-63

##### Adiponectin gene expression in human granulosa cells of women with PCOS

Artimani T<sup>1</sup>, Aflatoonian R<sup>2</sup>, Saidijam M<sup>3</sup>, Amiri I<sup>4</sup>, Yavangi M<sup>4</sup>, Shabab N<sup>3</sup>, Ashrafi M<sup>2</sup>, Mehdizadeh M<sup>1, 5</sup>.

1. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, Tehran, Iran.
3. Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
4. The Research Center for Endometr and Endometriosis, Hamadan University of Medical Sciences, Hamadan, Iran.
5. Department of Anatomy, School of Medicine, Cellular and Molecular Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Email: mehdizadehm@tums.ac.ir

**Introduction:** Several studies have recently discovered that many factors from adipose tissue can effect and regulate the reproductive system. Adiponectin is the most abundant protein secreted by white adipose tissue, and its plasma levels are inversely related to body mass index. In addition, adiponectin modulates steroid synthetic protein gene expression and it is possible to interact with both LH (luteinizing hormone) and insulin to induce the expression of cyclooxygenase-2 transcripts in granulosa cells. However, conflicting results regarding association of adiponectin levels in women with polycystic ovary syndrome (PCOS) have been reported. In current study, the level of adiponectin mRNA in granulosa cells in PCOS patients was investigated.

**Materials and Methods:** After approval of Tehran University of medical sciences ethics committee and written informed consent of the patient, follicular fluid was collected from patients undergoing oocyte retrieval. 10 patients were divided into two groups, PCOS and control according to Rotterdam criteria. A series of isolation and purification methods was performed, including density gradient centrifugation, MACS (use of antibody bead complexes) and RNA extraction. RT-PCR was applied to show the existence of adiponectin gene in granulosa cell and quantitative real-time PCR

analysis was applied to investigate the relative expression of this gene in purified granulosa cells.

**Results:** The expression of adiponectin was significantly different between case and control groups, with the lowest expression in the PCOS group. Hypoadiponectinaemia was more evident in obese women with PCOS when compared with non-PCOS counterparts.

**Conclusion:** In conclusion, it appears that adiponectin either acting directly on ovulation or by increasing sensitivity to insulin and gonadotropins, influences the ovulatory process.

**Key words:** Adiponectin, BMI, Granulosa cell, Polycystic ovary syndrome.

#### P-64

#### Polymorphism of cytochrome P450 2D6 (CYP2D6) gene in infertile women with PCOS

Modaresi-Nejad M<sup>1</sup>, Tarahomi N<sup>1</sup>, Shiva M<sup>2</sup>, Afsharian P<sup>3</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Endocrinology and Reproductive Health, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for reproductive Biomedicine, ACECR, Tehran, Iran.

Email: pafshar@royaninstitute.org

**Introduction:** Clomiphene is one of the most important drugs that used as the first step in ovulation induction of women with Polycystic Ovary Syndrome (PCOS). Clomiphene is metabolized by CYP2D6. Cytochrome P450 2D6 is an important factor in the metabolism of drugs used clinically. CYP2D6 gene is polymorphic with diverse distributions of allelic variants in different population. Different isotypes of CYP2D6 protein have different enzymatic activities which are including: Extensive Metaboliser (EM), Intermediate Metaboliser (IM), Ultrarapid Metaboliser (IM) and Poor Metaboliser (PM). 90% of the chromatin basic proteins during the steps of. The aim of our study was to define allelic variants of CYP2D6 gene in PCOS patients in comparison to 2 other groups used.

**Materials and Methods:** Blood samples were obtained from 40 infertile women referred to Royan Institute. Sampling was completed for 40 healthy fertile women and 40 women under ART treatment (referred as male factor) as control groups. PCR- RFLP analysis was applied on extracted DNA to detect polymorphism of CYP2D6 gene.

**Results:** We identified 27.5% of all women in our study shown P34S polymorphism. G42E was not detected in any of our samples.

**Conclusion:** PCOS patients and ART control groups who had P34S polymorphism failed to Clomiphene therapy.

**Key words:** Polycystic ovary syndrome, CYP2D6, Restriction fragment length polymorphism.

#### P-65

#### FMR1 premutation survey; prominent etiology for premature ovarian failure

Eslami A<sup>1</sup>, Eslami H<sup>1</sup>, Asadpour O<sup>2</sup>, Totonchi M<sup>2</sup>, Madani T<sup>3</sup>, Yaghmaei P<sup>1</sup>, Shahhosseini M<sup>2</sup>, Hourabi H<sup>2</sup>, Mohseni Meybodi A<sup>2</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: anahitamohseni@gmail.com

**Introduction:** Premature ovarian failure (POF) refers to ovaries loss of function in approximately 1% of women under the age of 40 and is one of the major causes of female infertility. It is characterized by amenorrhea, low levels of estrogen and high levels of FSH. The known causes of POF can be classified as infections, ovariotoxic chemotherapy, pelvic surgery, autoimmune disease and genetic factors. Among the latter, POF can be associated with abnormalities affecting X chromosome such as fragile X syndrome associated to premutation in FMR1 (50- 200 CGG repeats). Investigation of the FMR1 premutation prevalence in women with POF.

**Materials and Methods:** A questionnaire including data related to reproductive status of patient such as age, menstrual cycle status and hormonal profile (FSH and AMH levels) was completed for each patient. 79 blood samples were collected from women with POF referred to Royan Institute under their informed consent. 30 women with proved fertility and normal hormonal profile enrolled as control group. DNA was extracted from blood samples by salting out method. The repeated trinucleotide sequence was amplified by hot start PCR and run on 4% agarose gel over night. Different DNA bands were purified from the gel and number of Trinucleotide Repeats were determined by sequencing.

**Results:** The mean serum FSH level was high  $102 \pm 25.85$  (>40 IU/L), and the mean serum AMH level was low  $0.63 \pm 0.25$  (<0.3 ng/mL) in individuals with POF. 13 (16%) of 79 women with POF had FMR1 premutation, compared with 0 (0%) of 30 control.

**Conclusion:** According to our results, determining the number of CGG repeats in FMR1 gene can be used as a diagnostic method in the susceptible individuals. Thus, screening the FMR1 premutation may help them choose between oocyte freezing or earlier pregnancy.

**Key words:** FMR1 gene, CGG repeats, Premature ovarian failure.

#### P-66

#### Comparative analysis of effects of vaginal isosorbide mononitrate pill and low-dose syntocinon for cervical ripening in childbirth

Tabatabaie RS<sup>1</sup>, Dehghani Firouzabadi R<sup>2</sup>, Farajkhoda T<sup>3</sup>.

1. Department of Obstetrics and Gynecology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Research and Clinical Center for Infertility, Shahid



Sadoughi University of Medical Sciences, Yazd, Iran.

3. Department of Midwifery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: farajkhoda\_t@yahoo.com

**Introduction:** Cervical ripening for childbirth is one of the midwifery problems. Nitric oxide-releasing medicines have been recently taken into account, regarded as Endothelium-dependent loosening agents and an important biological medium in human body. Isosorbide mononitrate pill vaginally used for cervical ripening appears in 20 mg and also 40 mg doses in majority of research works.

**Materials and Methods:** This study was conducted to determine effect of isosorbide mononitrate pills and then compare it with low-dose syntocinon. Statistical society of the current study consists of first-time pregnant women admitted in Shahid Sadoughi Yazd Hospital in 2007-2008. 100 primigravida women were randomly divided into two groups of receiving 40 mg isosorbide mononitrate pill vaginally (50 persons) and low-dose syntocinon (50 persons). Impact and safety of both methods were compared for two groups in terms of cervical ripening and duration between treatment start and childbirth. The data were analyzed using SPSS (version 15) software by precise statistical tests of Fischer and t-student.

**Results:** In both groups, no significant difference was observed in terms of variables of average age, and pregnancy age (sonography, first day of last menstruation period). Average duration of cervical ripening until childbirth induction was  $36.13 \pm 4.05$  hours for 40 mg isosorbide mononitrate pill group and  $36.28 \pm 3.88$  hours for low-dose syntocinon group ( $p=0.876$ ). There was no significant difference between two groups regarding type of childbirth (caesarian and vaginal childbirth). The most prevalent medicine side effects in isosorbide mononitrate pill and low-dose syntocinon groups were slight headache (70%) and tachysystole/hypertonus (4%), respectively. Apgar scores of first and fifth minutes were the same in both groups.

**Conclusion:** Administration of vaginal isosorbide mononitrate pill has an influence on cervical ripening similar to low-dose syntocinon and might be used as an effective, simple, inexpensive, and safe method in cervical ripening process.

**Key words:** Isosorbide Mononitrate, Low-dose Syntocinon, Cervical Ripening, Childbirth.

## P-67

### Effect of pretreatment of ovine sperm on male pronuclear formation and normal fertilization following intracytoplasmic sperm injection

Derakhshan-Horeh M, Shirazi A, Ahmadi E, Nazari H, Heidari B.

1. Research Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran.
2. Department of Reproductive Biotechnology Reproductive Biotechnology Research Center, Avicenna Research

Institute, Tehran, Iran.

Email: mh\_derakhshan@yahoo.com

**Introduction:** During the final stages of mammalian sperm maturation, their nuclear structure becomes progressively condensed and stabilized by the formation of disulfide bonds. Therefore one of the problems in sheep ICSI is the failure or rarely decondensation of sperm chromatins, resulting in a low rate of male pronuclear formation and normal fertilization in artificially activated ovine oocytes matured in vitro. In view of the dependence of sperm nuclear decondensation on the reduction of disulfide bonds, the aim of the present study is to determine how far the efficiency of ovine ICSI (judged by the rate of male and female PN formation and subsequent embryo development) can be improved by sperm pretreatment with DTT, SDS and two-time frozen/thawed. In addition, considering the role of detergents in solubilizing proteins and disruption of sperm membrane, the probable synergistic effect between DTT and SDS on MPN formation is evaluated.

**Materials and Methods:** Sperm were incubated in HSOF-BSA medium containing: I) 5 mM DTT for 20 min, II) 5 mM DTT plus 0.1% SDS for 20 min, and III) 0.1% SDS for 20 min. The sperm were then washed twice in BSA-HSOF and prepared for injection in the same medium. In group IV) after swim up, the sperm without cryoprotectant was transferred to the -20 freezer for 5 min and then plunged directly into liquid nitrogen for 1 min, and thawed immediately in 37°C. This procedure (freezing in liquid nitrogen and thawing at 37°C) was carried out two times. The non-treated sperm served as control. In pretreated and control groups the sperm were prepared in HSOF-BSA for injection.

**Results:** The rate of swollen sperm head in SDS group (N=63) was higher ( $p<0.05$ ) than DTT and SDS+DTT groups (44.4% vs. 13% and 13.2%, respectively). The proportion of intact sperm head in DTT group (N=96) was higher ( $p<0.05$ ) than other SDS and SDS+DTT groups (44% vs. 9.5 and 17%, respectively). Sperm head decondensation (male pronucleus formation) in SDS+DTT (N=53, 58.5%) was higher than SDS (27%) and DTT (38.5%) groups ( $p<0.05$ ) except for the oocytes injected with frozen-thawed (N=64, 45.3%) sperm.

**Conclusion:** In the current study pretreatment of sperm with DTT+SDS led to the highest rate of MPN formation among ICSI oocytes. It seems DTT (an agent that specifically reduces disulfide bonds [ $S=S$ ]) and SDS (an anionic detergent which disrupts non-covalent bonds) could exert their synergistic effect on MPN formation through sperm nuclear decondensation and sperm membrane damage, respectively.

**Key words:** ICSI, Dithiothreitol, Ovine, Development.

## P-68

### Inactivating FSH receptor mutations are not associated with premature ovarian failure in Iranian patients

**Ghezelayagh Z<sup>1</sup>, Asadpour O<sup>2</sup>, Eslami A<sup>3</sup>, Totonchi M<sup>2</sup>, Zari Moradi Sh<sup>2</sup>, Gourabi H<sup>2</sup>, Mohseni Meybodi A<sup>2</sup>.**

1. School of Developmental Biology, University of Sciences and Culture, ACECR, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.

**Email:** anahitamohseni@gmail.com

**Introduction:** Immaturity of the ovarian follicles results in an infertility citation called POF or Premature Ovarian Failure. Women with this disorder lack graffian follicles so they don't have any oocytes for ovulation and therefore go through menopause before the age of 40 and have high levels of gonadotropin hormones (FSH & LH). On the other hand, Follicle stimulating hormone has a critical role in the maturation of the ovarian follicles from the antral to the graffian stage.

**Materials and Methods:** FSH will start a signaling cascade in the granulosa cells after sitting on its receptor, FSH receptor, which its activation will lead to follicle To investigate whether mutations on FSH receptor are involved in POF disorder, two main inactivating mutations were The presence of two mutations 566C>T and 1555C>A were analyzed in a case control study comprised of 40 POF patients and 40 control samples which all were Iranian women who had referred to Royan Institute. Firstly their karyotype and FMR1 gene were checked to be normal. At the second stage, the DNA of peripheral blood samples was amplified by two pair of primers. For determining allelic variant status, RFLP, SSCP and Sequencing were done on the amplified PCR products.

**Results:** All the control and case samples had the normal GCA and CCC genotype; hence no inactivating mutations (GTA & ACC respectively) were seen in Iranian POF patients. The FSH mean in the blood test of these patients were 52.5 IU/ml.

**Conclusion:** Although these mutations, especially 566C>T were seen in other populations, this study showed that FSHR gene inactivating mutations are not frequent in Iranian POF patients. We suggest studying other inactivating mutations and polymorphisms of FSHR gene in Iranian POF patients maturation.

**Key words:** Follicle Stimulating Hormone Receptor, polymorphism, Premature Ovarian Failure, SSCP.

## P-69

### **Comparison between CA125, CA19-9 and CA15-3 in ovarian cancer and endometriosis in the patients with ovarian masses**

**Abdollahifard S, Danaii Sh, Faturachi F.**

Department of Obstetrics and Gynecology, Alzahra Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.

**Email:** danaiish@gmail.com

**Introduction:** The gold standard for diagnosis of endometriosis is histological conformation with laparoscopy but there is more desire to diagnose it

clinically or paraclinically with markers. One of the most useful markers is CA<sub>125</sub> but as it is fluctuant in most gynecologic complications its level is important in differential diagnosis of ovarian cancer from endometriosis. The objective of the present study was to comparison the concentrations of CA<sub>125</sub>, CA<sub>15-3</sub>, CA<sub>19-9</sub>, in patients with ovarian endometrioma and ovarian cancer.

**Materials and Methods:** This is a descriptive-inferential study. 100 women with ovarian masses were divided into two groups; group A consisted of 50 patients with ovarian cancer, and group B consisted of 50 women with ovarian endometrioma all women's serum analysed about the level of CA<sub>125</sub>, CA<sub>15-3</sub> and CA<sub>19-9</sub>. After the review of questionnaires, the data were analyzed by SPSS- 13 software.

**Results:** The concentration of CA-125 in serum of the patients with ovarian cancer was significantly higher than patients with endometriosis but for CA 19-9 and CA<sub>15-3</sub>, serum concentrations were not statistically different between these two groups.

**Conclusion:** These data confirmed that CA 125 is the most important marker in the diagnosis of ovarian cancer and endometriosis although the level of CA<sub>125</sub> in ovarian cancer was higher. Variations detected in CA 19-9 and CA 15-3 had no statistical significance between 2 group of patients with endometriosis and ovarian cancer.

**Key words:** Ovarian cancer, Endometriosis, Tumor marker.

## P-70

### **Effect of Omega-3 supplementation on gonadotropins and prolactin levels in women with polycystic ovary syndrome: a double blinded randomized controlled trial**

**Daneshbodi H, Vaziri N, Nadjarzadeh A, Lotfi MH, Mozaffari- Khosravi H.**

1. Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biostatistics and Epidemiology, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

**Email:** azadnajarzadeh@ssu.ac.ir

**Introduction:** Polycystic ovary syndrome (PCOS) is a multifactorial, complex genetic, endocrine, and metabolic disorder. Some studies show the effect of omega-3 on PCOS treatment but its effect on sex hormone in this group of patients is unknown. Evaluating the influence of omega 3 supplementation on some gonadotropins and prolactin in PCOS women.

**Materials and Methods:** A randomized double blind placebo-controlled clinical trial was conducted on 84 women with polycystic ovary syndrome diagnosed depending on Rotterdam Criteria; referred to the fertility and infertility research center and Shahid Sadoughi hospital in Yazd. Overweight and obese women between 20-40 years with PCOS randomly assigned to take capsules of Omega-3 (3 g/d) or placebo for 8 weeks. Serum levels of LH, FSH, and prolactin were

measured before and after the intervention. The LH/FSH ratio was calculated.

**Results:** After the supplementation the mean of LH was decreased about 1.74mIU/ml ( $p<0.005$ ) in omega 3 group, but there was not a significant change in placebo ones ( $p=0.87$ ). The mean of LH/FSH ratio was decreased about 0.76 ( $p<0.005$ ) in supplement group, however FSH and prolactin level were not changed significantly.

**Conclusion:** The results of our study showed that Omega-3 treatment was associated with significant improvement in LH concentration and LH/FSH ratio in PCOS. This improvement can cause the improvement of reproductive system.

**Key words:** Polycystic ovary syndrome, Omega 3, LH, FSH, Prolactin.

## P-71

### Factors affecting live birth rate in in-vitro fertilization/ intracytoplasmic sperm injection cycles: role of number of embryo transferred

Arabipoor A<sup>1</sup>, Ashrafi M<sup>1,2</sup>, Madani T<sup>1, 2</sup>, Karimian L<sup>3</sup>, Chehrizi M<sup>4</sup>.

1. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Department of Epidemiology and Reproductive Health at Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: arezoo.arabipoor@gmail.com

**Introduction:** The primary purpose was to investigate whether increasing the number of good embryos increases the overall pregnancy, live birth in women undergoing in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) cycles. The secondary objective was to evaluate the impact of maternal age on the outcome of IVF/ICSI.

**Materials and Methods:** Data from six hundred nineteen women with primary infertility that had at least one fresh embryo with good quality for transfer at each IVF/ICSI cycles in reproductive biomedicine research center, Royan Institute, Tehran, Iran in the period from September 2006 to June 2010 were evaluated retrospectively. Pregnancy outcomes were compared into four groups according to number of fresh embryos transfer.

**Results:** Our results showed a lower pregnancy rate (16%) in patients with one embryo transfer compared with the other groups ( $p=0.001$ ). Pregnancy rate in patients with two, three and four embryo transfers were similar. The rate of live births was significantly lower in single embryo transfer, compared to the other groups

( $p=0.001$ ) but it was similar between two, three and four embryos transfer groups ( $p= 0.6$ ). The rates of multiple pregnancies were (0%), (27%), (45.2%), (27.7%) respectively in four groups. Clinical pregnancy and live birth rates were similar between two, three and four embryos transfer groups in women younger and older than 33 years old thus reduction of the number of embryo transferred did not decrease the clinical pregnancy and live birth rates in both age levels.

**Conclusion:** Due to the impossibility of implementing the elective single embryo transfer technique in the most infertility centers in the world, we suggest transfer of double instead of triple or quadruple embryos, whereas at least one good quality embryo is available for transfer in women less than 40 years.

**Key words:** Number of embryo transfer, ICSI cycle, Live birth.

## P-72

### The association between MLH3 C2531T polymorphism and non-obstructive azoospermia in Iranian infertile men

Heidari Rostami HR, Mirfakhraie R, Movafagh A, Gholami M, Ahmadi H, Zare-Abdollahi D.

Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: reza\_mirfakhraie@yahoo.com

**Introduction:** Genetic factors cause about 10% of male infertility. However, the etiology of the majority of male infertility cases including non-obstructive azoospermia or severe oligospermia remains idiopathic. Defects in DNA repair during spermatogenesis are thought to underlie some types of testicular failure. Evidence is accumulating to demonstrate the importance of DNA repair defects in human non-obstructive azoospermia. In eukaryotes, homologs of the bacterial MutL proteins, including MLH3, function in DNA mismatch repair and recombination pathway and therefore may play a crucial role in spermatogenesis.

**Materials and Methods:** In the present study, we investigated the association between C2531T polymorphism in the MLH3 gene non-obstructive azoospermia using the tetra-amplification refractory mutation system-PCR (4P-ARMS-PCR) method in 94 non-obstructive azoospermia patients and 80 fertile controls. The amplified DNA was visualized on 2.0% agarose gel containing DNA safe viewer.

**Results:** We observed an increased risk of male infertility associated with the MLH3 (CT+TT) (OR, 2.34; 95% CI, 1.19-4.62;  $p=0.012$ ) genotype, compared to the MLH3 CC genotype.

**Conclusion:** Our results show an association between C2531T polymorphism in MLH3 with non-obstructive azoospermia. Therefore, the MLH3 polymorphism may be genetic determinants for human spermatogenesis impairment.

**Key words:** Non-obstructive azoospermia, MLH3, Mismatch repair.

### P-73

#### Efficacy of Histoprep® gradient for isolating ovine epididymal sperm

Shokrollahi E, Barati F, Gooraninejad S.

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Email: fabrtir@yahoo.com

**Introduction:** Presence of dead sperm, somatic cells or bacteria within low quality semen can decrease its fertility. Different techniques are used for separation of motile sperm including: swim-up, percoll gradient, glass-wool filtration and sephadex filtration. Swim-up and swim-down based techniques are routine for isolating motile sperm. The most applicable swim-down technique for sperm isolation is continuous or discontinuous gradient of percoll. However other high density materials, e.g., iodixanol, iohexol, also were used for isolating motile sperms. Sodium diatrizoate is a hyper-osmolar compound, contrast radiographic imaging, that was successfully used for isolating and purification of different orders of blood cells. However, there is a report that systemic infusion of the materials may disrupt spermatogenesis in mice with no significant effect on sperm abnormalities. In vitro exposure of macrophages to this compound reduced their efficiency. Ficoll gradient was effective in isolating goat sperm. Recently, a combination of sodium ditrizoate with 5.6% (w/v) ficoll successfully isolated viable lymphocytes. The aim of the present study was to find efficacy of the new combination of sodium ditrizoate with ficoll on isolating ovine epididymal sperm.

**Materials and Methods:** Testes were provide from the local slaughterhouse and transported to the Laboratory on ice and stored in refrigerator for 24 hrs. An aliquot of (200 uL) of sperm suspension placed on the bottom of a conical tube then 800 uL of washing solution was added very slowly. The tube placed in a humified (95%) atmosphere of 38°C with and 5 % of CO<sub>2</sub> for 30-45 min. Finally, 200 µL above of the suspension was removed and evaluated for concentration and estimating sperm progressive motility. Histoprep, a high density medium, composed of a radiographic contrast medium, sodium ditrazoate, and Ficoll (5.6% v/v) which is efficient in isolating pure viable human lymphocytes. An aliquot of sperm suspension (200 µL) overlaid on 1 mL of Histoprep then centrifuged with 1000 g for 10 min. The pelleted sperm in the bottom of the tube was removed and mixed with washing medium (5 mL) and centrifuged at 200 g for 5 min. The supernatant was removed and pellet was mixed in washing medium (1 mL) and analyzed for progressive motility and sperm concentration. Counted sperms in both procedures were adjusted to have a concentration of  $2 \times 10^6$  (sperm/mL) by mixing equal volumes of the washing and the fertilization media, and drops of 75 µL were prepared under mineral oil and incubated in a CO<sub>2</sub> incubator for 30-45 min for capacitation. Ovine ovaries were provided from the local slouterhouse and the matured

oocytes were co-incubated with sperm in IVF medium for 5 h, and the zygotes were transferred to the embryo culture medium that it had being changed every 48 h to evaluate cleavage (On day 3 post insemination) and blastocyst (on day 6 post insemination) rates.

**Results:** The mean retrieved sperm from epididymides was  $468.5 \times 10^6$  that showed a significant individual variation. Mean isolated motile sperm by swim-up and Histoprep were 23.3 and 29.4 ( $\times 10^6$ ), respectively. The proportion of isolated sperm to total retrieved sperm was significantly different between swim-up and Histoprep methods. Mean estimated percentage of progressive motile sperm before processing was  $73 \pm 3.43$ . The mean percentages of progressive motility of isolated sperm were  $84 \pm 3.18$  and  $87.7 \pm 1.98$  for Histoprep® and swim-up methods, respectively. The mean proportions of post to pre processing sperm motility were not significantly different between Histoprep® ( $1.14 \pm 0.03$ ) and swim-up ( $1.22 \pm 0.04$ ) methods ( $p > 0.05$ ). Mean percentages of cleaved zygotes were  $62 \pm 5.21$  and  $64.4 \pm 12.35$  for Histoprep and swim-up methods, respectively ( $p > 0.05$ ). the percentage of embryos that reached blastocyst was not significantly different between two methods of sperm isolation (Histoprep:  $6.8 \pm 1.1$  and swim-up:  $7.9 \pm 1.21$ ;  $p > 0.05$ ).

**Conclusion:** The results of the present study showed efficacy of Histoprep continuous gradient as well as swim-up for isolation of ovine motile epididymal sperm followed by in vitro fertilization.

**Key words:** Sheep, Swim up, Histotrep, Embryo development.

### P-74

#### Role of 17-b oesteradiol for induction of regulatory T cells

Fatemi R<sup>1</sup>, Vahedian Z<sup>2</sup>, Mirzadegan E<sup>1</sup>, Mahdavi pour M<sup>1</sup>, Zarnani AH<sup>3</sup>, Jeddi-Tehrani M<sup>2</sup>, Idali F<sup>1</sup>.

1. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: R.Fatemi@avicenna.ac.ir; F.Ideali@avicenna.ac.ir

**Introduction:** Estrogens are providing protection for the semi-allogeneic fetus during pregnancy. Recent investigations have shown that during pregnancy, the elevated number of regulatory T cells (Tregs) correlates with 17-β oestradiol (E<sub>2</sub>) level. Treg activity, through production of cytokines, may then affect the pregnancy out come. The aim of this study was to evaluate the level of cytokine expression in co-culture experiment whit E<sub>2</sub>-conditioned T cells together with autologous and allogeneic PBMC.

**Materials and Methods:** We treated the magnetic bead separated peripheral blood naïve T cells (n=4) with E<sub>2</sub> and anti-CD28 antibody in anti-CD3 coated plates in presence or absence of E<sub>2</sub> for 96h at 37°C and 5% CO<sub>2</sub>. Two different concentrations of E<sub>2</sub> (pregnancy and preovulatory) were used. Naïve T cells with no

treatment were termed as control cells. The phenotype of induced Tregs was investigated through analysis of CD25 and Foxp3 expression. ELISA technique was applied for measurement of cytokines: IL-10, TNF- $\alpha$  and IFN- $\gamma$  in cell supernatants of co-culture of conditioned T cells with autologous and allogeneic PBMC in the presence or absence of PHA.

**Results:** After 4 days, the induction of CD25 and Foxp3 was detected by flow cytometry. E<sub>2</sub>-conditioned T cells showed significantly higher potential to release IL-10 in co-cultures with autologous and allogeneic PBMC than control cells. Significant differences were served in the level of IL-10 when E<sub>2</sub>-conditioned at both preovulatory and pregnancy concentrations co-cultured with allogeneic PBMC in comparison to TCR conditioned T cells in presence of PHA. Decreased TNF- $\alpha$  and IFN- $\gamma$  and IFN- $\gamma$ /IL-10 ratio in co-culture with auto PBMC were noted especially in cultures with preovulatory concentration of E<sub>2</sub> as compared to TCR and E<sub>2</sub> pregnancy-conditioned T cells. In addition, production of TNF- $\alpha$  by E<sub>2</sub> preovulatory in co-culture with auto PBMC was significantly lower than that by E<sub>2</sub> pregnancy in the absence of PHA. In supernatant of all conditioned T cell groups co-cultured with both autologous and allogeneic PBMC was found higher IFN- $\gamma$  level than corresponding control cells.

**Conclusion:** Our data shows the potential roll of 17 $\beta$ -oestradiol at preovulatory concentration as a good candidate for invitro generation of Tregs, which may contribute to developing novel therapeutic methods for treatment of autoimmune, may reduce abortion incidence in the future.

**Key words:** Regulatory T cells, 17-b oestradiol, Preovulatory, Pregnancy.

## P-75

### A meta-analysis of using intravenous immunoglobulin (IVIG) for treatment of recurrent miscarriage

Najafi S.

Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: soheil.najafi@gmail.com

**Introduction:** Recurrent miscarriage is a reproductive problem that affects 1% of couples. Despite different treatments many of these couples remain childless. One of the immunological treatments is intravenous immunoglobulin (IVIG) that which is human IgG prepared from pooled plasma. In this study, we evaluated the therapeutic effects of intravenous immunoglobulin in treatment of recurrent miscarriage.

**Materials and Methods:** Meta-analysis of published randomized controlled and cohort-controlled trials of evaluating IVIG treatment of recurrent miscarriage in PubMed data center.

**Results:** The particular benefits of IVIG therapy are in reducing recurrent miscarriage in women with initially elevated peripheral blood NK cells. IVIG produced a significant improvement in the live birth rate in women

with recurrent miscarriage who had initially elevated NKT cells and NK cells. Furthermore, IVIG has been shown to favorably decrease Th1/Th2 ratios and at a clinical level appears to be helpful for women with recurrent miscarriage and elevated preconception Th1:Th2 ratios.

**Conclusion:** Intravenous immunoglobulin therapy for women with recurrent miscarriage was a safe and beneficial therapeutic strategy that associated with high clinical pregnancy and live birth rates.

**Key words:** Recurrent Miscarriage, Intravenous Immunoglobulin, Meta-Analysis, Immunotherapy.

## P-76

### Saccharin consumption increases sperm apoptosis in adult mice

Rahimipour M, Talebi AR, Omid M.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: marziehrahimipour@yahoo.com

**Introduction:** Saccharin is an artificial non-caloric sweetener that first synthesized in 1879. It is used to sweeten products such as drinks, candies, biscuits, medicines, and toothpaste whereas our bodies cannot metabolize it. Sodium saccharin is considered as an important factor in tumor promotion, induces urinary bladder tumors in male rats but not in humans. However, according to our knowledge, there is no data on the effects of saccharin on reproductive performances especially sperm fertility potential. Since sperm DNA has a critical role in reproductive function.

**Materials and Methods:** Totally 14 adult male mice were divided to 2 groups. Group 1 served as control fed on basal diet and group 2 received water containing saccharin (0.2% w/v) as experimental group for 35 days. Finally, left cauda epididymis was cut and placed in Ham's F10. Swimmied-out spermatozoa were used to analyze Sperm DNA integrity by means of two different tests; SCD (sperm chromatin dispersion) and TUNEL assay.

**Results:** Following saccharin consumption, we had an increased rate of sperm DNA damage and apoptosis in experimental group when compared with control one. In spite of this effect, in saccharin group the external genitalia showed anatomical alterations (inflammation) in 4 animals (57%) and atrophy in external genitalia and urinary bladder tumor in one (14%).

**Conclusion:** According to our results, saccharin consumption may increase the rate of sperm apoptosis in mice.

**Key words:** Sperm, Chromatin, Apoptosis, Saccharin, Mice.

## P-77

### The role of TLR3, NOD1 and NOD2 in diabetes-induced inflammation: Implications for testis complications

Dehghani Firoozabadi A<sup>1,2,3,5</sup>, Ebrahimi B<sup>1</sup>, Shojaei S<sup>5</sup>, Sheikhhah MH<sup>4</sup>, Haghparast A<sup>2,3</sup>, Forouzannia SKh<sup>1</sup>.

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2. Department Pathobiology, Immunology Section, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
  3. Department of Pathology, Biotechnology Section, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
  4. Department of Genetics, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
  5. Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.
- Email: ali.dehghani.cardio@gmail.com

**Introduction:** Type 1 diabetes is a condition affecting the pancreas that is chronic. It is characterized by insufficient amounts of insulin being produced by the pancreas, resulting in blood glucose levels that are higher than normal. Male infertility is defined as the sperm being immobile or misshapen, not enough sperm being produced, or there being a blockage present resulting in the delivery of sperm being prevented. Chronic health problems, such as type 1 diabetes, can cause male infertility. Type 1 diabetes and male infertility can go hand in hand. For a long time now, it has been assumed that type 1 diabetes may cause infertility in men. In recent years, this link has been further explored and links have been discovered. The nucleotide-binding oligomerization domain receptors, in short NOD-like receptors (NLRs) are a class of pattern recognition receptors (PRR) that respond to host perturbation from either infectious agents or cellular stress. NLRs can cooperate with Toll-like receptors and regulate inflammatory and apoptotic response. The function of most NLR family members and TLRs has not been characterized and their role in diabetic testis immune responses remains unclear. We hypothesized that diabetic type 1 condition is accompanied by enhanced endogenous pattern recognition receptors expression such as Toll-like receptor 3 (TLR3), NOD1 and NOD2 in diabetic testis. Although recently a few studies have shown that these genes are induced in diabetes, it is not clear whether these gene are involved in the development of diabetes and diabetic infertility. In this study the time course expression of the TLR3, NOD1 and NOD2 genes in testis tissue of diabetic male Wistar rats were studied.

**Material and Methods:** Diabetes type 1 (T1D) was induced in male Wistar rats with intraperitoneal (I.P.) single injection of Streptozotocin. At first, to evaluate the effect of diabetes on testis cellular density, histological studies were performed on testis tissue. In different time points (4, 6, 8= early phase of diabetes progression and 20 weeks= late phase of diabetes progression) post diabetes type 1 induction, rats were euthanized and total testis tissues was removed for further analysis. Total RNA was extracted from testis tissues samples followed by cDNA synthesization using Random-hexamer primers. Exon specific TLR3, NOD1 and NOD2 primers were used to amplify rat TLR3, NOD1 and NOD2 cDNA. After performing semiquantitative RT-PCR, the expression levels of TLR3, NOD1 and NOD1 mRNA was quantified by real

time quantitative PCR (qPCR).

**Results:** Our histological results showed a reduced cellular density and apoptosis in testis tissues following diabetes type 1 induction. Also, molecular analysis showed up-regulation of TLR3, NOD1 and NOD2 transcripts during the time course after diabetes induction especially 20<sup>th</sup> week, as compared to the control group.

**Conclusion:** The abundant expression of TLR3, NOD1 and NOD2, the major components of innate immunity system, provides strong evidence that these receptors may play important roles in the pathogenesis and expansion of diabetes and it is possible that the expression of these pattern recognition receptors eventually lead to infertility in male Wistar rats.

**Key words:** Testis, Type1 Diabetes, TLR3, NOD1, NOD2, Wistar rats.

## P-78

### Various pattern of CC chemokine expression in term and pre-term neonates along with their respected mothers

Fattahpour Sh<sup>1</sup>, Moogooei M<sup>2</sup>, Aminzadeh F<sup>3</sup>, Ghorashi Z<sup>4</sup>, Khorramdelazad H<sup>5</sup>, Hassanshahi Gh<sup>5</sup>.

1. Department of Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
2. Department of Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
3. Department of Gynecology Surgery, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
4. Faculty of Nursing and Midwifery, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
5. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

Email: ghassanshahi@rums.ac.ir

**Introduction:** The pathology of pre-term delivery is mostly unknown but is a frequent disorder, worldwide. Previous studies revealed the involvement of chemokines in Pre-term infancy. This project aimed to detect the circulating levels of CCL2, CCL5 and CCL11 in term and pre-term delivered neonates and their respected mothers.

**Materials and Methods:** Cord and peripheral blood samples were collected from 53 pre-term and 53 term neonates along with their Serum levels of CCL2, CCL5 and CCL11 were measured by ELISA and their mRNA levels were detected by real time PCR. The demographic parameters were also collected by a questionnaire.

**Results:** Present results indicated elevated levels of CCL2 in mothers with pre-term delivery and their respected neonates. Although our results demonstrated that CCL5 was elevated in mothers with pre-term pregnancy but this chemokine was undetectable in their corresponding neonates, however, it was detected in term neonates. We also observed decreased of CCL11 in mothers with pre-term neonates, however, this chemokine was inversely increased in pre-term neonates

in compare to term neonates.

**Conclusion:** our results are indicative for the fact that chemokine in cord and mothers obtained here could possibly applied as marker for rapid detection of pre-term either due to inflammatory responses or other leading causes observed before delivery. These type of accurate laboratory based examination of early pre-term delivery is also valuable for optimal monitoring of the most favorable pregnancy outcome and to minimize related risks in mother and fetus.

**Key words:** Pre-term, Term, Delivery, Neonate, Chemokine, CCL2, CCL5, CCL11.

#### P-79

### Intravenous administration of mesenchymal stem cells improve functional recovery after traumatic brain injury in rats

Anbari F<sup>1</sup>, Khalili MA<sup>1</sup>, Bahrami AR<sup>2</sup>, Aflatounian B<sup>3</sup>, Khoradmehr A<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biology, Sciences Faculty, Mashhad University, Mashhad, Iran.
3. Madar Hospital, Yazd, Iran.

Email: fateme\_anb2010@yahoo.com

**Introduction:** Traumatic brain injury (TBI) is a major cause of mortality worldwide. The clinical studies have proven cell therapy as a major option to improve the brain function post trauma, and by possible regeneration of the nervous system. The aim was to investigate the role of intravenous administration of Mesenchymal Stem Cells (MSCs) after TBI.

**Materials and Methods:** The animals were divided into two groups of TBI + PBS (control) and TBI + MSC (experimental). TBI was done based on model of Foda-Marmarou. MSCs were exposed with bromodeoxyuridine (BrdU) 48 h before intravenous injection. The experimental group received 3×10<sup>6</sup> MSCs, labeled with BrdU, and PBS was injected to control group, into the lateral tail vein, 24 h after TBI. The neurological severity score (NSS) was performed to evaluate the neurological function at 0, 1, 7 and 14 days after TBI. MSCs migration and their differentiation to neurons and astrocyte cells were examined with immunohistochemistry.

**Results:** Results from NSS showed no significant differences between the groups of control and experimental at 1 and 7 days (3.5±1.41 vs 5.63±2.44, p=0.06) and (2±1.69 vs 3.62±1.99, p=0.06), respectively. However, motor deficits decreased significantly in the experimental rats when compared with control group at 14 days (0.75±0.7 vs 2.75±1.83, p=0.01). Immunohistochemical studies showed that BrdU positive MSCs migrated via venous system to the cerebral tissue. Also, migrated MSCs to the injured brain were able to express neuronal (NeuN) and astrocytes (GFAP) markers.

**Conclusion:** Intravenous administration of MSCs seems to improve the functional recovery and neural cells

regeneration after TBI in animal model. MSCs application may be suitable as therapeutic strategy for regeneration of CNS after TBI technique.

**Key words:** Mesenchymal stem cell, Traumatic brain injury, Immunohistochemistry technique.

#### P-80

### Recommended foods for female infertility in Iranian traditional medicine

Nejabatbakhsh F<sup>1</sup>, Bioos S<sup>1</sup>, Siahpoosh MB<sup>1</sup>, Sohrabvand F<sup>2</sup>.

1. Department of Iranian Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.

Email: Nejabatbakhsh@razi.tums.ac.ir

**Introduction:** Infertility, defined as the inability to conceive a child after 1 year of having intercourse without using contraception, affects 10–15% of couples. Causes of female infertility and their treatments are expressed in classical medicine and Iranian Traditional Medicine (ITM). ITM physicians stressed the importance of nutrition in the prevention and treatment of diseases. Also in this problem recommended many foods for each causes. The aim was to explore the causes of female infertility and recommended foods for treating this problem addressed through the ITM original resources.

**Materials and Methods:** Specific data related to the subject among all referral ITM texts was extracted firstly, and then the collected data were analyzed using inductive content analysis.

**Results:** The analysis of data revealed that female infertility in ITM has two causes; physiologic and psychosomatic. Signs and symptoms and recommended drugs and foods for physiologic causes are expressed, that in this study stressed the recommended foods.

**Conclusion:** Foods that can enhance fertility can be recommended to female patients who suffer from infertility in medical centers to aid in their treatment.

**Key words:** Female infertility, Nutrition, Iranian traditional medicine.

#### P-81

### Simple vaginal medicaments for unexplained infertility in Iranian traditional medicine

Nejabatbakhsh F<sup>1</sup>, Bioos S<sup>1</sup>, Sohrabvand F<sup>2</sup>.

1. Department of Iranian Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.

Email: Nejabatbakhsh@razi.tums.ac.ir

**Introduction:** Unexplained infertility accounts for 30% of all infertility among couples. Iranian Traditional Medicine (ITM) stressed the different medicaments in the treatment of unexplained infertility. Iranian traditional physicians have categorized these medicaments into 2 groups; oral and non-oral, for

example vaginal medicaments. The aim was to explore the vaginal medicaments recommended by ITM scientists in treatment of unexplained infertility addressed through the ITM original resources.

**Materials and Methods:** In this review study specific data related to the subject among all referral ITM texts was extracted firstly, and then the collected data were analyzed using inductive content analysis.

**Results:** The analysis of data revealed that different vaginal medicaments for unexplained infertility have been stressed in referral ITM texts. This study provided five vaginal medicaments that are composed from simple materials.

**Conclusion:** Vaginal medicaments that can enhance fertility can be recommended to female patients who suffer from unexplained infertility in medical centers.

**Key words:** Iranian Traditional Medicine, Vaginal medicaments, Unexplained infertility.

## P-82

### Combination of thrombophilic gene polymorphisms as a cause of increased risk of recurrent pregnancy loss

Torabi R, Zarei S<sup>2</sup>, Jeddi-Tehrani M<sup>2</sup>.

1. Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Email: mahjed@yahoo.com

**Introduction:** Recurrent pregnancy loss (RPL) is a heterogeneous condition. While the role of acquired thrombophilia has been accepted as an etiology for RPL, the contribution of specific inherited thrombophilic gene polymorphisms to the disorder is still controversial. This study aimed to investigate 11 thrombophilic gene polymorphisms (Factor V LEIDEN, Factor V 4070 A/G.

**Materials and Methods:** One hundred women with a history of two or more consecutive abortions and 100 women with at least two live births and no miscarriages were included in the study and evaluated for the presence of these 11 polymorphisms using PCR-RFLP technique. The data were statistically analyzed using Mann-Whitney test and logistic regression model.

**Results:** There was no relation between factor XIII 103G/T gene polymorphism with increased risk of RPL. However, the other 10 gene polymorphisms were found to be individually associated with increased/decreased risk of RPL. We found correlation between: FV Leiden and FV A4070G, FXIII A614T and FXIII C1694T, and between MTHFR C677T and MTHFR A1298C with RPL by Spearman test. Multiple logistic regression model for analyzing the simultaneous effects of these polymorphisms on the risk of RPL showed that six of these 11 polymorphisms (Factor V 1691G/A, Factor V

5279A/G, Factor XIII 614A/T,  $\beta$ -Fibrinogen -455G/A, ITGB3 1565T/C, and MTHFR 1298A/C) together were associated with increased risk of RPL.

**Conclusion:** It is possible to calculate the risk of abortion in a patient with RPL by determining only six of the 10 polymorphisms that are individually associated with RPL.

**Key words:** PCR-RFLP, Recurrent pregnancy loss, Thrombophilic gene.

## P-83

### The higher rate of SNP of H2BFWT gene in an Iranian population with idiopathic spermatogenesis impairment

Rasti M<sup>1</sup>, Kalantar SM<sup>2</sup>, Sheikhha MH<sup>2</sup>.

1. International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: majidrasti64@yahoo.com

**Introduction:** Spermatogenesis is a complex process including spermatogonial stem cell proliferation, meiosis and spermatid differentiation. Genetic variation of those genes involved in this process may play an important role in impaired spermatogenesis. Histones are the major nuclear proteins in eukaryotic cell nuclei that are responsible for the nucleosome structure of chromatin. The H2B family, member W, testis specific (H2BFWT) gene encodes a testis specific histone that plays a crucial role in reorganization and remodeling of chromatin and epigenetic regulation during spermatogenesis, suggesting that the gene may be involved in spermatogenesis impairment.

**Materials and Methods:** To test the speculation, the allele and haplotype frequencies of one single-nucleotide polymorphism loci in this gene, -9C>T is investigated in 100 infertile patients with idiopathic azoospermia or oligozoospermia and 100 fertile men as controls using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay.

**Results:** As the results, the frequencies of -9T is significantly higher in patients than those in controls; after stratifying patients, the significant higher frequencies is still detected in allele -9T for azoospermia. The haplotype CA was significantly decreased whereas TG was significantly increased in infertile patients compared with controls.

**Conclusion:** These results indicated that the polymorphism -9C>T in H2BFWT gene are associated with male infertility with idiopathic azoospermia or oligozoospermia, suggesting that H2BFWT gene might be contribute to susceptibility to spermatogenesis impairment in Iranian population.

**Key words:** H2BFWT, Single-nucleotide polymorphism, Male infertility, Spermatogenesis impairment.



**P-84**

**Is there any difference between ART outcome of too young patients and adult patients?**

**Yari N, Etebary S, Khalili MA.**

*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** nahidyari@yahoo.com

**Introduction:** It is well known that with increasing age, fertility decreases in woman. There is a question; does very young age affect fertility? Since, some of the patients who undergo ART treatments are very young patients or young donors, fecundity investigation seems necessary in this group. Studies have shown that after age of 35 years, reproductive function is strongly influenced. However, only a few comprehensive studies on the effect of very young age on the fertility potential have been reported lately. The aim was to evaluate the outcome of ART in very young infertile women in the range of 17 to 25 years.

**Materials and Methods:** Data from patients who underwent IVF/ICSI over 20 years from 1992-2012 that referred to Yazd infertility Center were analyzed retrospectively. The records of 407 infertile patients aged 17-25 years (study group) and 407 infertile patients aged 26-35 years (control group) were collected and reviewed. In groups, demographics characteristics, clinical data, details of the ART treatment cycles and ART outcome were compared between the two groups.

**Results:** In study group the number of oocytes obtained 9.25, the number of MII oocytes 7.9, the number of cleaved embryos in IVF cycle 7.58 and in ICSI cycle 4.35, fertilization rates in IVF cycles 82% and in ICSI cycles 55.8% and chemical pregnancy rate ( $\beta$ hCG<sub>+</sub>) was 25.8%. In control group the number of oocytes obtained 7.8, the number of MII oocytes 6.57, the number of cleaved embryos in IVF cycle 4.4 and in ICSI cycles 3.28, fertilization rate in IVF cycles 53%, in ICSI cycles 49.61% and chemical pregnancy rate was 29.02%. The number of follicles greater than 14 mm ( $p < 0.001$ ), the number of oocytes obtained ( $p < 0.001$ ), the number of MII oocyte ( $p < 0.001$ ), the number of cleaved embryos in IVF cycle ( $p < 0.001$ ) and in ICSI cycle ( $p < 0.001$ ) was significantly higher in the study group. While chemical pregnancy rate was not different between the two groups.

**Conclusion:** Pregnancy rate in young infertile women was higher than very young infertile women. It seems that the clinical ART outcomes are similar among infertile patients under age of 36 years.

**Key words:** *In vitro fertilization, Young patient, Pregnancy, Reproductive outcome.*

**P-85**

**Comparison of single embryo transfer with multiple embryo transfer in the different age groups of infertile patients**

**Etebary S, Yari N, Khalili MA.**

*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

**Email:** etebary.s@gmail.com

**Introduction:** Many studies have been done to identify the predictors of ART outcomes. The most obvious variables for this purpose are female age and number and quality of transferred embryos. Traditionally, assisted reproductive treatment has been associated with a 20-fold rate of multiple pregnancies compared with spontaneous twin pregnancies. On the other hand obstetric, neonatal, developmental and financial consequences represent the main iatrogenic complications of ART program. The only method to limit the number of twins is to transfer only one embryo (SET). This retrospective study compared pregnancy outcomes from a single embryo transfer (SET) with multiple embryo transfer (MET) in very young women ( $\leq 25$  years) and young women ( $> 25$  years).

**Materials and Methods:** Data from infertile patients who referred for ART treatment to Yazd infertility center during 1992 to 2012 were studied retrospectively. Records of patients with SET or MET were studied in two groups:  $\leq 25$  (375 patients), and  $> 25$  years (346 patients). Demographic data including age, job, place of residency, clinical information, including cause and duration of infertility, type of infertility and kind and number of treatment, details of ART treatment cycles consisting number of antral follicles, number and grade of oocyte, semen analysis, number and grade of embryos, number of transferred embryos, and pregnancy outcomes were compared.

**Results:** In  $\leq 25$  years group the mean number of follicles  $> 14$  mm 11.03, the number of oocyte 9.71, the number of MII oocyte 8.38, and the grade of transferred embryos was 17.19. In  $> 25$  years group the mean number of follicles  $> 14$  mm 9.26, the number of oocyte 8.45, the number of MII oocyte 7.23, and the grade of transferred embryos was 16.99. The pregnancy rates from SET  $\leq 25$  years group was 15.8% and from MET was 29.4%. In  $> 25$  years group, the pregnancy rates from SET was 11.5% and from MET was 39.3%.

**Conclusion:** The pregnancy outcomes with SET or MET weren't different in very young infertile women. So, it is better to perform SET to avoid multiple pregnancy complications.

**Key words:** *Single embryo transfer (SET), Multiple embryo transfer (MET), Pregnancy outcome.*

**P-86**

**A study on walnut effect on reproductive system of adult male rats**

**Ghorbani R, Mokhtari T, Bakhtiari M, Jalili C, Khazaei M.**

*Department of Anatomy, Kermanshah University of Medical Sciences, Kermanshah, Iran.*

**Email:** rostamgh@yahoo.com

**Introduction:** Diabetes mellitus is a degenerative disease that has deleterious effects on male reproductive

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

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

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

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

**Key words:** Walnut, Diabetes, Streptozotocin, Infertility.

## P-87

### Epidemiologic study of male related infertility in patients of Kermanshah Infertility Treatment Center

Bakhtiari M<sup>1</sup>, Kamravamanesh M<sup>2</sup>, Esfandiari M<sup>2</sup>, Poursalman M<sup>3</sup>, Yousefi V<sup>3</sup>, Seyedi T<sup>4</sup>.

1. Department of Anatomy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
2. Department of Midwifery, Kermanshah University of Medical Sciences, Kermanshah, Iran.
3. Kermanshah University of Medical Sciences, Kermanshah, Iran.
4. Department of Psychology, Kermanshah University of Medical Sciences, Kermanshah, Iran.

**Email:** mbakhtiari@kums.ac.ir

**Introduction:** Our current knowledge of the epidemiology of infertility is limited and outdated. It is known that, male infertility is identified as a common cause of infertility. This study was done to investigate the incidence of male factor and the most common cause of male infertility in patients of Kermanshah infertility treatment center in Mo'tazedi Hospital with both primary and secondary infertility.

**Materials and Methods:** Data were studied from 102 patients with secondary infertility and 313 patients with primary infertility (age between 20 and 48 years) who were referred to Kermanshah Infertility treatment

Center (Iran) during March 2011 until April 2012. Analysis of data performed by SPSS.

**Results:** Our results showed that, the male factor in 50.5% of patients with primary infertility and 47.3% of them with secondary infertility was known as the cause of infertility. In the patients with male related primary infertility, 23.5% azoospermia, 41.3% spermogram disorders, 3.03% congenital disorders, 16.7% varicocele, 2.6% infectious and immunological diseases and 12.8% idiopathic causes were reported. In the patients with male related secondary infertility, 24.1% azoospermia, 47.5% spermogram disorders, 2.5% congenital disorders, 7.3% varicocele, 8.6% infectious and immunological diseases and 12% idiopathic causes were reported.

**Conclusion:** This study showed that in both primary and secondary infertile patients, incidence of male factor were more than normal reported. It may be due to recent environmental changes such as influx of haze and dust in the west of Iran.

**Key words:** Epidemiology, Primary infertility, Secondary infertility, Male factor.

## P-88

### Effect of maternal exposure to methamphetamine in different pregnancy periods in mice

Khoradmehr A, Anvari M, Golzadeh J, Mirjalili F, Sabour M.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

**Email:** mehrarezoo@gmail.com

**Introduction:** Methamphetamine (METH) is a neurotoxic drug and addictive central nervous system stimulation which is widely abused by pregnant women. METH crosses through the placenta. Clinical studies showed that exposure to METH during pregnancy, was effect on fetal development in humans. In the present study, we were evaluated the histological changes of mice fetal brains which were exposed (Meth) in uterus during different pregnancy period.

**Materials and Methods:** We were used thirty two NMRI female mice (8-12 week old) and were divided to four groups. The first and second groups of pregnant mice were injected METH subcutaneously at a dose of 10 mg/kg/day during gestational day (GD), from 1 to 7 and 7-14 days respectively. The third group was administered METH at similar dose during 1 to 14 days and control group was received saline. On GD 14, fetuses of all groups were getting out and weighted. Crown-rump length of fixed fetuses in formalin and circumference of head fetuses were measured with caliper and were evaluated abnormal morphology by Hematoxylin-Eosin staining.

**Results:** We found various types of morphological damages in METH fetal brains, including exencephaly, cleft palate and hemorrhage and in some cases, also were observed premature fetals. Only fetuses in forth

group was showed lower body weight compared to control group significantly ( $p<0.001$ ). Maternal body weight was not significant difference between groups. Measurements showed Length of fetal body size in forth group was significantly decreased in first group compared to control group ( $p<0.05$ ), head circumference in forth group not shown significant difference compared other groups.

**Conclusion:** We concluded that METH use during pregnancy can cause histological brain alterations in fetuses, which neurotoxicity might be related to reactive oxygen species and can damage development of brain fetus. Lower body weight gain, it could be caused by the pharmacological action of METH, which increases the metabolism and decreases appetite. The toxicity mechanisms of METH are not well characterized in humans.

**Key words:** Methamphetamine, Pregnancy, Mice, Brain.

#### P-89

#### The value of prenatal ultrasound in the diagnosis of obstructing kidney diseases postnatal; determination of a cut-off point level for postnatal referral

Nafisi-Moghadam R, Shishesaz B.

Department of Radiology, Shahid Sadoughi Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: nafisi@ssu.ac.ir; nafisi.moghadam@gmail.com

**Introduction:** To establish guidelines for postnatal referral of fetuses presenting with mild pyelectasis in the second trimester of pregnancy.

**Materials and Methods:** In our study during a period of one year, for a total number of 2319 pregnancies, 81 pregnancies were identified as having a renal abnormality on a routine late second trimester scan. This group of patients was booked to our clinic for routine pregnancy ultrasound (US) examination. Prenatal sonographic fetal anteroposterior diameter of  $\geq 4$  mm in the second trimester, which persisted to  $\geq 8$  mm in postnatally were selected as the inclusion criteria. The maximum degree of dilation observed antenatally for each fetus as well as any associated abnormality which was also recorded. A detailed scan was performed by a radiologist at the late second to third trimester. The follow up antenatal renal tract US was performed at the same clinic and by the same radiologist. Neonatal pediatric investigations using US examination were performed at 6 weeks antenatally. Further investigation on infants who had abnormalities in the latter step underwent immediate micturating cystourethrography (MCUG). Intravenous urograph (IVP) were used in conjunction with US at 6 weeks to assess obstruction if needed, particularly for planning surgery. MCUG was performed in those with unobstructed system, persisting pelvicalyceal dilation or a suggestion of reflux which included the finding of a dilated ureter either antenatally or postnatally. Children with normal renal tracts were discharged from our list.

postnatal outcome was measured by the clinical finding(s), radiological investigation, biochemical measurements. Renal size and growth were measured by using US technique.

**Results:** In our study, from a total of 2319 pregnancies, 9 infants had abnormalities postnatally. In this group, 5 infants (55.6%) were male and 4 infants (44.6%) were female, giving a male: female ratio of 1.25. In regard to outcome, 69 of male and female fetuses with pyelectasis had normal calices size postnatally. Although fetal pelvicalyceal dilation is more common in males, the outlook in terms of renal prognosis appear similar for both sexes. A cut-off level of 8-mm is found with a sensitivity and specificity of 90% and 100% respectively.

**Conclusion:** Prenatal US diagnosis may cause parental anxiety, especially when the significance of the finding and hence long term prognosis is uncertain. However with non lethal renal anomalies, uterodetection allows through early investigation and treatment in the postnatal period which may reduce long term childhood morbidity. In this study, after establishing a diagnosis of mild pyelectasis at late second trimester, a cut-off level of 9 mm nearly covers our cases but it has a low specificity but includes most of pathology. A cut-off point of 8 mm detects most significant pathology, but with a higher level of specificity, although VUR may not be detected.

**Key words:** Pyelectasis, Ultrasonography, Prenatal, Postnatal.

#### P-90

#### Evaluation effect of intrauterine human chorionic gonadotropin injection before embryo transfer in implantation and pregnancy rate in infertile patients and comparison with conventional embryo transfer in IVF/ICSI/ET cycles

Janati S<sup>1,2</sup>, Dehghani Firouzabadi R<sup>1</sup>, Mohseni F<sup>1</sup>, Razi MH<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Dezful University of Medical Sciences, Dezful, Iran.

Email: Sjanati@gmail.com

**Introduction:** Successful implantation after in vitro fertilization (IVF) and embryo transfer (ET) depends on numerous factors associated to the endometrial receptivity and the embryo quality. Implantation is exact complex process that is regulated by various factors which one the most important is human chorionic gonadotropin (hCG). The aim of this prospective randomized controlled study is investigation of the effect of intrauterine human chorionic gonadotropin (HCG) injection before embryo transfer On pregnancy outcome in infertile couples.

**Materials and Methods:** We evaluated 159 patients undergoing IVF/ICSI with antagonist protocol at our center for treatment of infertility. Patients divided into three groups using table of random numbers. In cases,

case group I (n=53) received 500 IU of HCG and case group II, (n=53) received 1000 IU of HCG intrauterine injection before ET, and control group received nothing before ET.

**Results:** There were no significant differences between groups in implantation rate, chemical and clinical pregnancy rate and abortion rate ( $p>0.05$ ).

**Conclusion:** Our results showed Intrauterine injection of hCG before embryo transfer (ET) does not improved the implantation and pregnancy rates in IVF/ICSI /ET cycles.

**Key words:** Intrauterine HCG injection, IVF/ICSI, Embryo transfer, Pregnancy outcome.

## P-91

### Comparison of effect of transdermal estradiol and estradiol valerate on endometrial receptivity in frozen-thawed embryo transfer cycles

Janati S<sup>1,2</sup>, Davar R<sup>1</sup>, Mohseni F<sup>1</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Obstetrics and Gynecology, Dezful University of Medical Sciences, Dezful, Iran.

Email: Sjanati@ymail.com

**Introduction:** Implantation and pregnancy establishment depend on the interactions between embryo development and endometrial receptivity. Frozen-thawed embryo transfer (FET) has been successfully performed in natural and artificial cycles as prepared with exogenous steroids (oral or transdermal). The aim of this prospective randomized clinical trial, was comparing clinical outcome of two method of endometrial preparation in frozen-thawed embryo transfer cycles by oral estradiol and 17 $\beta$ -estradiol transdermal patch to select the optimal endometrial preparation protocol.

**Materials and Methods:** Total number of 90 patients that nominated for frozen -thawed embryo transfer. In study group (n=45), 17-B estradiol transdermal patches 100 mcg applied since second day of menstrual cycle and continue every other day each patch was removed after four days .In control group (n=45), for endometrial preparation at the same time of cycle oral estradiol 6 mgr daily started.

**Results:** There were no significant differences of demographic characteristics between two groups .There are significant differences in estradiol level on day of progesterone administration and day of embryo transfer between two groups ( $p=0.001$ ) but no significant different were observed between them in biochemical, clinical pregnancy rate (32.6 % vs. 33.3%,  $p=1$ , 30.2% vs. 33.3%,  $p=0.81$  respectively). There were no differences in implantation rate or spontaneous abortion rate either.

**Conclusion:** Our results showed in spite of no significant different in biochemical and clinical pregnancy rates in two groups but due to reduces costs, minimum dose of estradiol transdermal patches,

simplified protocol and increases patient compliance we can used instead of oral estradiol in Frozen-thawed embryo transfer cycles.

**Key words:** Frozen-thawed embryo transfer, E<sub>2</sub> supplementation, Estradiol patches, Endometrial preparation.

## P-92

### Differential expression of CXC chemokines CXCL1, CXCL9, CXCL10 and CXCL12 in semen and serum of infertile and fertile men

Abedinzadeh M<sup>1</sup>, Fatehi F<sup>2</sup>, Abedinzadeh M<sup>3</sup>, Assar Sh<sup>4</sup>, Khoramdelazad H<sup>2</sup>, Hakimi H<sup>4</sup>, Kazemiarababadi M<sup>4</sup>, Hassanshahi Gh<sup>2</sup>.

1. Department of Urology surgery, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
2. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
3. Yazd Jahad Daneshgahi University, Yazd, Iran.
4. Immunology of Infection diseases Research center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

Email: abedinoro@yahoo.com

**Introduction:** The predominance of male genital tract inflammations is a main debatable issue in male infertility. Chemokines are a subgroup of cytokines with several biological properties vary from recruitment of leukocytes to the sites of injury, infection and inflammation to angiogenesis, and angiostasis. There are also growing evidences showing that chemokines are present in the reproductive tract. Seminal plasma also contains various members of chemokines family including CXCL1, CXCL9, CXCL10, and CXCL12. Due to the fact that infertile men may involve with inflammatory conditions, this study was aimed to examine and compare both serum and semen levels of these chemokines in fertile and infertile men.

**Materials and Methods:** In this experimental study, the semen samples in parallel with peripheral blood specimens were harvested from 68 consecutive patients aged 30-45 years at the Molecular Medicine Research Center, Rafsanjan, Iran. To detect and quantify the aforementioned chemokines in aliquots of frozen-thawed seminal plasma and serum, the ELISA method was applied.

**Results:** The results of this study indicated that except CXCL12, the seminal levels of the other three chemokines in infertile men were significantly lower than those in fertile men. Inversely, findings of present study indicated that all of studied CXC chemokines were significantly elevated in serum of infertile men in compare to fertile ones.

**Conclusion:** Due to the important role played by these ckemokines in chemotactic response of cell systems, it could possibly be concluded that the spermatozoa motility would be reduced, following decreased levels of these mediators of chemotaxis. On the other side, elevated levels of these chemokines in serum level may probably reveal, the development of a inflammatory

response, mediated by chemokines, which may be involved in pathogenesis of men infertility.

**Key words:** CXCL1, CXCL9, CXCL10, CXCL12, Infertility, semen, Serum.

#### P-93

### The effect of laser assisted hatching (LAH) on the rates of implantation and pregnancy in good prognosis patients

Kiani K<sup>1,2</sup>, Ashrafi M<sup>1,3</sup>, Akhlaghi A<sup>4</sup>, Zolfaghari Z<sup>1</sup>.

1. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Obstetrics and Gynecology, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Epidemiology and Reproductive Health at Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

**Email:** kiani.kiandokht@gmail.com; k-kiani@razi.tums.ac.ir

**Introduction:** To assess the effect of laser assisted hatching (LAH) on the rates of implantation and pregnancy in good prognosis patients who had no more than one previous unsuccessful IVF-ET attempt.

**Materials and Methods:** In this retrospective study, 535 good prognosis patients undergoing their first or second unsuccessful IVF/ICSI cycles were included between October 2009 and August 2010. In the patients who were selected for LAH, on the day 2 or 3 after ovum pick up, just before embryo transfer, ZP was drilled using a 1.48 micrometer wave length infrared laser. The control group had conventional ICSI without laser drilling. The primary outcome measures were implantation and pregnancy rates.

**Results:** From the total of 535 patients, 166 patients underwent laser assisted hatching and 369 others had routine ICSI without LAH. The implantation rate was significantly lower in the laser hatched group compared with the non-LAH (16.2% vs. 23.7%, respectively;  $p=0.003$ ). The patients with LAH also had significantly lower pregnancy rate compared with the non-LAH (29.2% vs. 39.7%,  $p=0.022$ ). Three cases of abortion (0.6%) occurred in the patients who had no LAH; however, there was no abortion in the LAH group. The rate of multiple pregnancies was similar between the two groups (29.2% in LAH vs. 26.1% in Non-LAH,  $p=0.655$ ).

**Conclusion:** The results showed that laser hatching may decrease the chance of implantation and pregnancy rate in good prognosis patients. Therefore, the routine performance of LAH in good prognosis patients who had only one previous IVF failure should be unwarranted.

**Key words:** Laser assisted hatching, Good prognosis patients, Implantation rate, Pregnancy rate.

#### P-94

### Effect of time interval between sperm exposure with PVP on fertilization and embryonic development in human

Golestan Jahromi M, Khalili MA, Halvaei I.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

**Email:** golestanm48@yahoo.com

**Introduction:** Exposure of sperm to PVP has recently been found to cause submicroscopic changes in sperm structure; the sperm nucleus appeared to be damaged. The PVP-induced nuclear damage may have been due to breakdown of sperm membranes. Furthermore, suggested that PVP delayed the onset of calcium oscillations and sperm decondensation in the oocyte. Consequently, it is likely that exposure of sperm to PVP may suppress embryonic development. There are apparently no detailed reports regarding the specific effects of PVP on fertilization rate and embryonic development in humans.

**Material and Methods:** The objective of this study was to investigate the effects of polyvinylpyrrolidone (PVP) and hyaluronidase on fertilization rate and embryonic development. In the present study, 358 oocytes from 58 cycles were evaluated. We recorded the time interval between exposure of sperm with PVP and ICSI insemination, also we recorded the time of exposure of oocytes with hyaluronidase, and then each oocyte was put it in a single droplet of G1 media. All of the procedures were performed by a single embryologist over 6 month's period of time. Post-ICSI rates of fertilization and blastomere number as well as embryo quality were assessed. Data was analyzed by linear regression.

**Results:** No statistically significant differences were found between the time of exposure of oocytes to hyaluronidase with Blastomere number, embryo quality and Fertilization rate, but there are significant differences between the time of exposure of sperms with PVP and Fertilization rate ( $p=0.002$ ), Embryo quality ( $p=0.03$ ) and Blastomere numbers ( $p=0.001$ ).

**Conclusion:** In conclusion, different time of exposure of sperm with PVP can affect subsequent cleavage rate and embryonic development, so it is important to decrease the time of sperm exposure to PVP as possible.

**Key words:** Time interval, PVP, Hyaluronidase.

#### P-95

### Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat

Miresmaeili SM<sup>1,2</sup>, Halvaei I<sup>3</sup>, Fesahat F<sup>3</sup>, Fallah A<sup>3</sup>, Nikonahad N<sup>1</sup>, Taherinejad M<sup>4</sup>.

1. Yazd Academic Center of Education, Culture and Research Higher Education Institute, Yazd, Iran.
2. Department of Biology, Sciences Faculty, Zahedan, Iran.
3. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

4. Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Email: farzaneh.fesahat@gmail.com

**Introduction:** Nanoparticles have wide range of application while there rare studies regarding their probable effects on male reproductive system and spermatozoa. The aim was to evaluate the effect of different doses of silver nanoparticles (AgNPs) (70nm) on acrosome of rat spermatozoa and number of spermatogenic cells.

**Materials and Methods:** In this case-control study, in experimental group, 32 male Wistar rats (8 rats/group) received oral feeding AgNPs every 12 hr in one spermatogenesis period (48 days) by means of gavages in 25, 50, 100 and 200 mg/kg concentration (experimental groups 1-4). The control group (8 rats) was treated on schedule with distilled water. Spermatozoa were stained by triple staining protocol for acrosome reaction. Histological evaluation on testis sections was performed using tissue processing and hematoxylin-eosin (H&E) staining.

**Results:** There was significant difference between the control group and the experimental group 1 for acrosome reaction ( $11.00 \pm 0.00$  and  $24.25 \pm 3.68$ , respectively,  $p < 0.05$ ). There was only significant reduction in spermatogonia cells in experimental group 4. Experimental groups 2, 3 and 4 showed a significant reduction in number of primary spermatocytes and spermatids as well as spermatozoa. But there were no significant differences between different groups for Sertoli cell number and seminiferous tubule diameter.

**Conclusion:** It seems that Ag NPs, even in small size, have acute and significant effects on spermatogenesis and number of spermatogenic cells and also on acrosome reaction in sperm cells. More experimental investigations are necessary to elucidate better conclusion regarding the safety of nanoparticles on male reproduction system.

**Key words:** Silver nanoparticle, Sperm, Acrosome reaction.

#### P-96

#### Predicting value of high magnification sperm morphology evaluation in ICSI outcome

Ghazali Sh, Talebi AR, Khalili MA, Soleimani M, Razi MH, Halvaei I, Nabi A.

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: sh\_ghazali@yahoo.com

**Introduction:** Sperm quality can impact on early embryo development and pregnancy rate in intracytoplasmic sperm injection (ICSI) cycles, and sperm morphology as one of the principle parameters for predicting ICSI outcome has been addressed. To date, sperm morphology evaluation techniques have markedly improved, specially with introducing high magnification microscopy. The aim of this study was to assess value of high magnification sperm morphology evaluation in predicting ICSI outcome.

**Materials and Methods:** This prospective study was conducted on sperm samples from 117 patients who attended our center. After sperm processing by density-gradient technique, prepared spermatozoa were stained by Giemsa, and their nuclear vacuoles, shape and size were then analyzed at 6000 $\times$  magnification and classified into three classes according to their quality. Class I: spermatozoa having normal shape with no vacuoles, class II: spermatozoa having normal shape with vacuoles, class III: spermatozoa having abnormal shape with vacuoles. The rates of cleavage and pregnancy were assessed between different classes. The relationship between sperm morphology and progressive motility was also evaluated.

**Results:** The percentage of class I was significantly higher in positive pregnancy cases than the negatives (13.0% vs. 11.9%, respectively,  $p = 0.01$ ). No significant differences were obtained with respect to all sperm morphological classes with sperm progressive motility and cleavage rates.

**Conclusion:** It appears that sperm nuclear normalcy positively affects pregnancy rate in ICSI cycles. Therefore, assessment of sperm morphological characteristics using Giemsa staining at high magnification can be applied as a predictive tool in ICSI program.

**Key words:** Sperm morphology, High magnification, Giemsa staining.

#### P-97

#### Propofol or sodium thiopental in patients undergoing reproductive assisted technologies: comparative efficacy in hemodynamic recovery from anesthesia and outcome of oocyte retrieval

Jarahzadeh MH<sup>1,2</sup>, Dehghan Tezerjani M<sup>2</sup>, Musavi FS<sup>1</sup>, Behdad Sh<sup>2</sup>, Jouya R<sup>3</sup>, Soltani HR<sup>3</sup>.

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

2. Department of Anesthesiology and Critical Care, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

3. Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: dr.dehghan@gmail.com

**Introduction:** Thiopental sodium and Propofol are two widely-used drugs in the anesthesia process of ART induction. However, the side effects of the drugs on ART have not been identified yet. This study aimed at investigating the side effects, hemodynamic effects, and pregnancy outcomes of the drugs.

**Materials and Methods:** During this double blinded randomized controlled trial study, 90 patients underwent ART induction. The patients were assigned to two equal groups (each 45) with one receiving Thiopental and the other Propofol as the anesthetic. The Thiopental and Propofol groups were respectively induced with 5mg/kg of Thiopental Sodium and 2.5 mg/kg of Propofol. The entry hemodynamic parameters of the patients were documented. During the anesthesia process,

hemodynamic parameters were checked at five-minute intervals.

**Results:** The results of the study showed a statistically significant difference between the two groups in terms of their response to verbal stimulation ( $p<0.001$ ), the normalization time of the rate and quality of breathing ( $p<0.001$ ), nausea ( $p<0.001$ ), and vomiting ( $p<0.001$ ). Also, in comparison with the other group, all these parameters were better in Propofol group. There was found no significant difference between the two groups in terms of other variables.

**Conclusion:** Based on the findings of the study, the researchers used Propofol in cases where surgeries were required during the ART process. Additionally, the results of the study and the available literature suggest that Propofol has fewer known side effects.

**Key words:** Sodium Thiopental, Propofol, Assisted Reproductive technology.

## P-98

### Deleterious effect of geldanamycin on human sperm motility attenuated by trolox

Forootan Jahromi F, Keshtgar S, Ghani E.

Department of Physiology, Faculty of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran.

Email: fa.forootan@yahoo.com

**Introduction:** Geldanamycin (GA) is HSP90 specific inhibitor, and reduces sperm motility. It can also produce reactive oxygen species (ROS). It has been shown that ROS can reduce sperm viability and motility.

**Materials and Methods:** This examination was conducted to specify effectiveness of GA on sperm, is due to inhibition of HSP90 or it is due to producing of ROS. Normal samples ( $n=11$ ) were washed and treated with GA (10  $\mu$ M), trolox (200  $\mu$ M), and GA + trolox for 2 hours at 37°C under 5% CO<sub>2</sub>. Sperm motility was assessed before and during incubation (15, 30, 60, 90, 120 min). The percent of viable sperms determined at the beginning of experiment and also after 120 min incubation.

**Results:** The results showed that the GA reduced sperm motility in a time dependent manner and trolox reduced this negative effect on sperm motility. The percent of sperms with progressive motility in the presence of GA reached to  $52.86\pm5.85$  after 120 minutes, which was significantly lower than control ( $66.84\pm2.86$ ). The Percent of sperms which had progressive motility was  $63.47\pm3.05$ , and  $63.75\pm3.05$  in groups exposed to trolox, and trolox plus 10  $\mu$ M of GA, respectively. The percentage of live sperms decreased when the cells were exposed to GA. Trolox did not prevent this effect of GA.

**Conclusion:** With respect to the protective effect of trolox on sperm motility in the presence of GA, we conclude that the deteriorating effect of GA on motility is not because of Hsp90 inhibition, but may be due to ROS generation by GA. It seems that Hsp90 has a

protective effect on sperm survival, as it does on other cell types.

**Key words:** Heat shock protein 90, Geldanamycin, Human sperm, Trolox.

## P-99

### Effect of prolactin supplementation in freezing medium on the plasma membrane and acrosome integrity of human sperm after cryopreservation

Jabari A<sup>1</sup>, Dorostghoal M<sup>1,2</sup>, Hemadi M<sup>1</sup>, Kazeminejad SR<sup>3</sup>.

1. Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

2. Department of Anatomy, Faculty of Anatomy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

3. Department of Genetic, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Email: jabariayob@yahoo.com

**Introduction:** Sperm cryopreservation has become an important component of assisted reproduction. However, the use of cryopreserved human spermatozoa limits the success of these techniques because the freeze-thaw process results in reduced plasma membrane integrity, acrosome integrity and fertilization potential of sperm. In this regards, a variety of cryoprotective substances have been used in attempts to enhance human sperm resistance to the stress of cryopreservation. Prolactin has been identified in seminal plasma and recently its receptors have been found on spermatozoa. The purpose of this study was to evaluate the effects of prolactin supplementation to freezing medium on human sperm.

**Materials and Methods:** Ten semen samples of normospermic donors were prepared and split into six equal aliquots. Aliquots were mixed with freezing medium containing 0, 50, 100, 500 and 1000 ng/ml prolactin and treated samples were cryopreserved using slow freezing protocol in liquid nitrogen. After two week the samples were thawed and washed and then sperm membrane integrity and acrosomal integrity were analyzed.

**Results:** The results showed that addition of 100 ng/ml prolactin to freezing medium after thawing significantly improves membrane integrity and acrosomal integrity of human sperm. Also, the percent of membrane integrity of cryopreserved sperm increased significantly in 50 ng/ml prolactin-treated samples. However, No significance differences were seen in sperm membrane integrity and acrosomal integrity in samples treated by adding 1000 ng/ml prolactin in compared with control group.

**Conclusion:** It is concluded that addition of prolactin to freezing medium could improve post-thaw membrane integrity and acrosomal integrity of cryopreserved human sperm.

**Key words:** Sperm cryopreservation, Prolactin, Membrane integrity.



## P-100

### The prevalence of chlamydia trachomatis infection in infertile men with abnormal sperm parameters referred to Royan Institute

Moazenchi M<sup>1</sup>, Totonchi M<sup>2</sup>, Asadpor U<sup>2</sup>, Salman Yazdi R<sup>3</sup>, Haghighat S<sup>1</sup>, Sadighi Gilani MA<sup>3</sup>, Gourabi H<sup>2</sup>, Mohseni Meybodi A<sup>2</sup>.

1. Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: anahitamohseni@gmail.com

**Introduction:** Chlamydia Trachomatis (CT) is an obligate intracellular bacteria, that requires living cells to replicate itself. On average, 50% of men and 75% of women infected with CT are asymptomatic. CT infection can remain up to 4 years in the couple and affect their fertility. Chlamydia infection in men acts as a reservoir for transmission to women. Infection with CT can cause urinary tract inflammation, reduce sperm quality and acute epididymitis, especially in young men. The aim of this study was to determine the prevalence of CT infection in symptomatic and asymptomatic infertile men.

**Materials and Methods:** 800 semen samples of patients with abnormal spermograms were examined in order to detect the presence of CT. ELISA test was performed for presence of anti-CT IgA in these patients' semen plasma. Sperm's DNA was extracted in order to confirm the presence of CT. Genome amplification was performed using specific primers.

**Results:** Between the 800 patients with poor sperm parameters, 62 (8%) patients were diagnosed with Chlamydia infection. Among them 32 (52%) patients were symptomatic and 30 (48%) patients were asymptomatic.

**Conclusion:** Given the prevalence of infection and high frequency of asymptomatic patients among infertile individuals with poor sperm parameters, screening for infection among these patients can be essential in order to avoid the adverse effects.

**Key words:** Chlamydia trachomatis (CT), Men Infertility, Sperm parameters.

## P-101

### Stem cells from human dental pulp proliferate and express embryonic stem cell markers in serum free culture conditions

Ebrahimi B<sup>1</sup>, Yaghoobi MM<sup>2</sup>, Mohammadi Kamal Abadi A<sup>3</sup>.

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biotechnology, Research Institute for Environmental Sciences, International Center for

Sciences, High Technology and Environmental Sciences, Kerman, Iran.

3. School of Dentistry, Kerman Medical University, Kerman, Iran.

Email: Ebrahimi.b@ssu.ac.ir

**Introduction:** The growth of mesenchymal stem cells (MSCs) robustly depends on the culture conditions and requires medium supplemented with 10-20% fetal bovine serum (FBS), which is ill-defined and may possibly cause unfavorable immune reactions and this is a most important obstacle for their clinical utilization. In this study, we tested a serum-free  $\alpha$ -MEM on DPSCs to investigate their expansion and gene-expression analysis of embryonic markers despite other reports that use commercial serum-free media or regular media supplemented with serum substitutes such as protein C3, ULTROSER etc.

**Materials and Methods:** Dental pulp was extracted from third molars of healthy subjects. The pulp digested with collagenase/dispase (Roche) and released cells were cultured. The cells maintained in alpha-MEM, supplemented with %20 fetal bovine serum (FBS) (group 1) and without FBS (group 2), appropriate antibiotics and incubated in humidified incubator with %5 CO<sub>2</sub> at 37 °C. Total RNA was extracted using the RNeasy kit (Qiagen) treated with DNase I and first strand cDNA was synthesized using M-MuLV-RT enzyme. PCR was accomplished by primers specific for Nanog, Oct-4, Nucleostemin and  $\beta$ -actin using Taq DNA polymerase (Cinnagen) in each group separately. The PCR products were run on 1% agarose gel electrophoresis and visualized after EtBr staining.

**Results:** Interestingly, results showed that DPSCs survive and proliferate in serum-free alpha-MEM and express all examined embryonic stem cell markers Oct4, Nanog and Nucleostemin after two weeks of culture similar to that of the traditional culture; this behavior is not similar to other types of stem cells such as BMSCs.

**Conclusion:** These findings suggested that DPSCs are less dependent on FBS-related factors and could be easily propagated and differentiated in vitro. This could allow for identification of pathways involved in their stem cell biological characteristics. These results, suggested that these cells could provide a feasible option for stem cell-based therapies with no possible serum interferences.

**Key words:** Dental Pulp Stem Cells, Embryonic Stem Cell Markers, Nanog.

## P-102

### Prenatal testosterone exposure worsen the reproductive performance of male rat at adulthood

Ramezani Tehrani F<sup>1</sup>, Noroozzadeh M<sup>1</sup>, Zahediasl S<sup>2</sup>, Ghasemi A<sup>3</sup>, Piryaei A<sup>3</sup>, Azizi F<sup>4</sup>.

1. Reproduction Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Endocrine Physiology Research Center, Research Institute



for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

3. Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: zahedi@endocrine.ac.ir

**Introduction:** The reproductive system is extremely susceptible to environmental insults, for example exogenous steroids during gestational development and differentiation. Although there are several studies that demonstrated the impact of prenatal exposure of female fetuses to excess androgen, fetal programming of male reproductive system following androgen excess has not been well studied. We aimed to determine the effect of prenatal exposure of two different doses of testosterone on different gestational days.

**Materials and Methods:** Sixteen pregnant rats were randomly divided into two experimental groups and two control groups. Experimental group I were injected subcutaneously with 3 mg free testosterone on gestational days 16-19 and its controls were injected 500µl solvent for that time; experimental group II were injected subcutaneously with 20 mg free testosterone on day 20 of gestational period and its controls were injected 1 ml solvent at the same time. The reproductive system morphology and function of 32 male offspring of these study groups were compared at days 6-30-60 of age and after puberty.

**Results:** The anogenital distance (AGD) of the male offspring of both experimental groups had no significant differences on the different days of measurement, compared with controls. In the offspring of experimental group I, the testes weight, number of Sertoli cells, sperm count and motility and the serum concentration of testosterone after puberty were significantly decreased ( $p<0.05$ ); except for reduction of sperm motility ( $p<0.01$ ), the other effects were not observed in experimental group II.

**Conclusion:** It seems that the reproductive system in individuals exposed to high levels of androgens during fetal life should be evaluated at puberty, and likely to be treated.

**Key words:** Fetal life, Sperm quality, Sertoli cells, Testis.

### P-103

#### Evaluation of viability and morphological aspects of in vitro matured sheep oocytes in standard and biphasic systems

Vahabi Z<sup>1</sup>, Eftekhari-Yazdi P<sup>1</sup>, Dalman A<sup>1</sup>, Ebrahimi B<sup>1</sup>, Nematei AR<sup>2</sup>, Rajabpour Niknam M<sup>1</sup>.

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

2. Animal Core Facility, Reproductive Biomedicine Research Center, Royan Institute for Animal Biotechnology, ACECR, Tehran, Iran.

Email: peyazdi@yahoo.com

**Introduction:** In vitro maturation (IVM) is a technique that used to generate mature oocytes. There are two main (IVM) systems: Standard IVM and Biphasic IVM. In this study we evaluate the effects of two IVM systems on in vitro maturation of sheep oocytes.

**Materials and Methods:** Cumulus-oocyte complexes (COCs) were recovered from ovine ovaries collected at a local slaughterhouse. Good-quality COCs were randomly transferred into Standard (as group 1) and Biphasic IVM (as group 2) systems. In Biphasic system, first COCs were transferred to Pre-IVM medium supplemented with 100µM Forskolin and 500µM IBMX for 2 hours, then COCs were transferred to IVM medium supplemented with 20µM Cilostamide for 24 hours. After maturation period, oocytes were denuded from cumulus cells and MII oocytes with normal first polar body were counted. Oocyte viability was determined by means of the TUNEL technique.

**Results:** Oocytes matured in group 2 was significantly higher ( $93.4\pm1.8$ , 255 oocyte,  $p<0.05$ ) than group 1 ( $87.3\pm1.9$ , 224 oocyte). The percentage of viability among oocytes cultured in group 1 was  $99.1\pm0.9$  and was similar to that observed in group 2 oocytes ( $99.2\pm0.8$ ). Normal first polar body percentage in group 2 was significantly higher ( $43.2\pm3.7$ , 238 MII oocyte,  $p<0.0001$ ) than group 1 ( $10.3\pm2.3$ , 201 MII oocyte).

**Conclusion:** It seems that Biphasic IVM system by mimicking some characteristics of oocyte maturation in vivo could increase maturation rate and by synchronizing the cytoplasmic and nuclear maturation could increase normal first polar body in MII oocytes. MII oocytes displayed a similar viability regardless of the IVM system. It shown that our basic medium could protect oocytes from DNA degradation.

**Key words:** In vitro maturation, Sheep, Polar body, Apoptosis.

### P-104

#### Umbilical cord blood bank: Does it cover all ethnic groups of Iran based on HLA

Farjadian S<sup>1</sup>, Ebrahimkhani S<sup>2</sup>, Ebrahimi M<sup>2</sup>.

1. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran.

2. Department of Stem Cell, Royan Institute, Tehran, Iran.

Email: farjadsh@sums.ac.ir

**Introduction:** Because umbilical cord blood (UCB) allows transplantation of partially matched HLA grafts, it is considered a valuable resource for the treatment of hematologic malignancies and genetic diseases, especially for who lack of a compatible bone marrow donor. This study was designed to determine how many of ethnic groups of Iran can be covered by current public Royan UCB.

**Materials and Methods:** From 2009-2011, 4354 of all collected UCB samples (30%) met the necessary criteria for storage and were cryopreserved in public Royan UCB bank, Tehran, Iran. Parental demographic information was collected and a written informed consent has obtained from each family based on their

willingness to public donation of the UCB cells. HLA-A, B and DRB1 were typed for 1454 samples.

**Results:** The mean volume of the samples was  $83 \pm 40.7$  mL with a range of  $14-1200 \times 10^7$  nucleated cells in different samples. The most common HLA alleles were HLA-A\*2 (17%) and \*24 (15.4%); HLA-B\*35 (16.5%) and \*51 (13.3%), and HLA-DR\*11 (19.6%) and \*15 (14.4%). HLA-A\*24-B\*35-DR\*11 (1.9%), HLA-A\*02-B\*50-DR\*07 (1.9%), and HLA-A\*02-B\*51-DR\*11 (1.6%) were the predominant haplotypes.

**Conclusion:** Based on the broad therapeutic potential of hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), UCB can be considered the main source of different stem cells for cell-based therapy against various disorders. Based on HLA-DRB1 profiles, the current public Royan UCB bank can potentially be considered a proper resource for HSCs transplantation for Iranian recipients from Parsees and Zoroastrians. Regular educational programs for improving public knowledge about UCB advantages in transplantation can be effective to enrich this source as cover all Iranian ethnic groups in near future.

**Key words:** Stem cell transplantation, Umbilical cord blood, HLA.

## P-105

### Inflammatory infertility in rats treated chronically with L-arginine

Lakzaei F<sup>1</sup>, Karami M<sup>1,2</sup>, Jalali Nadoushan MR<sup>3</sup>, Hassani F<sup>1</sup>.

1. Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.
2. Neurophysiology Research Center of Shahed University, Shahed University, Tehran, Iran.
3. Department of Pathology, School of Medicine, Shahed University, Tehran, Iran.

Email: karami@shahed.ac.ir

**Introduction:** About 15 percent of married couples over the world suffer from infertility during breeding phase. Inflammatory events reason for a high percent of infertile women. This research discusses the occurrence of infertility in female rats treated L-arginine as a precursor of pro-inflammatory nitric oxide (NO).

**Materials and Methods:** The animals were female Wistar rats weighing 200-250g at the start of the experiments. They were kept as virgin to sustain them under the Diestrous phase of sexual cycle. The animals were intra-peritoneally (i.p.) injected the precursor of NO, L-arginine (50 mg/kg), throughout 9 days/ once a day. Another group of the rats were administered (i.p.) single anti-inflammatory naloxone (0.4 mg/kg, 9 days /once a day). The third group received naloxone (0.4 mg/kg, i.p.) 30 min priorly to the L-arginine (50 mg/kg). The control group solely received saline (1 ml/kg, i.p.) throughout the treatment period. After completion of the treatments, all rats were coupled with intact males. The females following observation of vaginal plaques were isolated and graded 0 of gestation. The females were

subsequently examined surgically in days 19-20 of gestation.

**Results:** Based on the fetus count result the L-arginine received group showed infertility compared with that of control. This rate was significantly upgraded by naloxone pre-treatment. The rate, however, indicated no significant change in single naloxone group. L-Arginine treated rats' ovaries moreover demonstrated polycystic characteristics in contrast to the control or naloxone group.

**Conclusion:** This study first records the infertility in rats chronically treated L-arginine. And likely concludes high rate infertility due to ovarian inflammatory events.

**Key words:** Infertility, Inflammation, L-arginine, Polycystic ovary, Fetus.

## P-106

### Evaluation of in-vitro fertilization outcome in long versus short insemination time protocol

Parsanezhad ME<sup>1</sup>, Matloob N<sup>2</sup>, Kooshesh L<sup>3</sup>, Zolghadri J<sup>4</sup>, Davari M<sup>2</sup>.

1. Division of Infertility and Reproductive Medicine, Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Embryology, Infertility Center, Dena Hospital, Shiraz, Iran.
3. Research Center, Shiraz Azad University, Shiraz, Iran.
4. Infertility and Reproductive Medicine Research Center, Shiraz University of Medical Sciences. Shiraz, Iran.

Email: parsame@rogers.com

**Introduction:** In-vitro fertilization is a process that oocytes and spermatozoa expose together in in-vitro conditions. In conventional IVF this exposure is between 16 to 20 hours incubation. Recent studies reported that prolonged exposure of gametes to each other may lead to toxic effects associated with reactive oxygen species (ROS) released by the spermatozoa. The aim of this study is to evaluate the microscopic aspect of short versus long insemination time protocols on embryo quality.

**Materials and Methods:** The present study was conducted on 30 infertile couples who would undergo in vitro fertilization. The female subjects' age was between 21 and 36 years old and the numbers of oocytes were considered between 10-15. After follicular puncture, the follicle content was placed in a special culture media and divided into two groups. Half of them were selected for short insemination and the other half were selected for long insemination. The fertilized oocytes in the short and long insemination culture media were separated after 3 and 16-20 hours respectively and moved to another medium including 30µl drops of cleavage. After 16-20 hours, the separated ovules following short and long insemination were investigated regarding fertilization and creation of male and female pronucleus (2PN) using an invert microscope. Then, in the second and the third days, the embryos were evaluated regarding the quality and number of blastomeres and fragmentation.

**Results:** The study results revealed a significant increase in the quality of the embryos in short insemination. ( $p<0.01$ ).

**Conclusion:** The qualitative changes observed in the current study showed that in order to have high-quality embryos in in vitro fertilization, it is better to perform short insemination.

**Key words:** *In vitro fertilization, Morphology, Fertilization, Cleavage, Embryo.*

#### P-107

##### **The study of adipose tissue derived stem cells proliferation rate by Ki-67**

**Ramezani M<sup>1</sup>, Ghorbanian MT<sup>1,2</sup>, Lashkarbolouki T<sup>1,2</sup>.**

1. *School of Biology, Damghan University, Damghan, Iran.*

2. *Institute of Biological Sciences, Damghan University, Damghan, Iran.*

**Email:** mt.ghorbanian000@gmail.com

**Introduction:** Adipose tissue-derived stem cells (ADSCs) are a promising cell source in regenerative medicine, because of its high proliferation rate in culture condition. Despite the undeniable usefulness of the BrdU method, but it has limitations and drawbacks. The possibility of BrdU producing mutated cells and the consequent severe abnormalities of the developing tissues has been reported. Given these drawbacks, an alternative method is highly desirable. Ki-67, a nuclear protein, which expressed in dividing cells for the entire duration of their mitotic process, was used in this study. Like BrdU, Ki-67 can be detected with immunocytochemistry. Unlike BrdU, Ki-67 is an endogenous marker that does not have any adverse effects on living cells.

**Materials and Methods:** Our main objective was to compare cell growth curve and rate, in ADSCs by ki-67 marker. We examined the differences in ADSCs were cultured in  $\alpha$ -MEM containing 10% fetal bovine serum, and 1% penicillin/streptomycin. The fourth passage of cells were plated on gelatinized slides at a density of  $5 \times 10^4 \text{ cm}^2$  and incubated at  $37^\circ\text{C}$  in an air atmosphere with 5%  $\text{CO}_2$ . The cells were immunostained by Anti ki-67, and then positive cells were counted at 24, 48, 72 and 96 h after culturing.

**Results:** The growth curve assay showed that the logarithmic phase of ADSCs elongated 48h. There was a significant increase of proliferation rate of ADSCs at 24 and 48 h compared with 72 and 96h.

**Conclusion:** ADSCs have a high proliferation rate at 24 h after culturing. Thus, Ki-67 is used as a proliferation marker and index.

**Key words:** *Adipose tissue, Derived stem cells, Proliferation rate, ki-67 immunostaining.*

#### P-108

##### **Effects of vitrification on maturation, fertilization and development in different developmental stages by cryotop method**

**Abedpour N, Rajaei F.**

*Department of Anatomy, Qazvin University of Medical Sciences, Qazvin, Iran.*

**Email:** nedaabedpour@gmail.com

**Introduction:** Results from previous studies regarding the effects of freezing on oocyte maturation, fertilization and embryonic development seem to be different. Given the importance of oocyte cryopreservation in clinic, the present study was aimed to investigate the effects of vitrification on oocyte maturation, fertilization and embryonic development in mouse oocytes by cryotop method.

**Materials and Methods:** Effects of vitrification on maturation, fertilization and development in different developmental stages by cryotop method. A total of 200 germinal vesicle (GV) and 200 metaphase II (MII) oocytes obtained from ovaries and fallopian tubes of NMRI mice respectively and divided into two control and experimental groups. Oocytes in experimental group were vitrified by Cryotop using vitrification medium (Origio) and were kept in liquid nitrogen for one month. After evaluating the survival rates of vitrified GV oocytes, they were cultured in maturation medium for 24h. In vitro maturation metaphase 2 (IVM-MII) and ovulated metaphase 2 (OV-MII) oocytes after insemination were assessed to hatching stage. The data was compared statistically using SPSS software and chi-square test.

**Results:** The incidence of maturation in vitrified and control GV oocytes showed significant decrease compared with the control group ( $p<0.05$ ). The fertilization rates of vitrified IVM-MII (44.1%) and OV-MII oocytes (50%) showed a significant decrease compared with the control group ( $p<0.05$ ) but there was no significant difference in developmental and hatching rates of both vitrified oocyte groups.

**Conclusion:** The results indicate that vitrification decreases maturation and fertilization rates in GV and MII mouse oocytes and vitrification may play a role in GV & MII oocyte injury.

**Key words:** *Vitrification, Maturation, Fertilization, Cryotop, Oocyte.*

#### P-109

##### **Assessment of ICSI outcome success rate for infertile couples with different cause of infertility**

**Jahanian Sadatmahalleh Sh<sup>1,2</sup>, Ashrafi M<sup>1</sup>, Akhoond MR<sup>3</sup>.**

1. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Centre, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*

2. *Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

3. *Department of Epidemiology and Reproductive Health at Reproductive Biomedicine Research Centre, Royan Institute for Reproductive Biomedicine, ACECR, Tehran-Iran.*

**Email:** shahideh.jahanian@modares.ac.ir

**Introduction:** Different success rate of ICSI (Intracytoplasmic Sperm injection) has been observing in various cause of infertility. In this study we evaluated the relation between ICSI outcome and special causes of infertility. We also aimed to examine parameters that might predict success following ICSI. Our prediction model has been developed in reproductive medicine to help gynaecologists in assessing the chances of pregnancy. With these models, gynaecologists can calculate the probability of a treatment pregnancy as well as the probability of success with ICSI.

**Materials and Methods:** In this cross sectional study, 1492 infertile women referred to Royan Institute between 2010 and 2011 were included. All of patients underwent ICSI cycles. Statistical Analysis was done using Chi-square and t-test. Logistic regression was done to build a prediction model in ICSI cycles. The performance of the model was calculated as the area under the receiver operating characteristic (ROC) curve. Calibration of the model was assessed by comparing the predicted probability of pregnancy in a category of patients and the observed percentage of pregnant woman in that category.

**Results:** The overall clinical pregnancy rate in our study was 33.9%. There was statistically significant difference in the Day 3 serum LH of the patients between the pregnant and the non pregnant groups ( $p < 0.05$ ). The mean day 3 serums FSH, TSH, PRL was no significant difference between groups ( $p > 0.05$ ). We did not find an association between cause of infertility and clinical outcomes ( $p > 0.05$ ). The number of metaphase II oocytes, embryo transfer, number of good embryo, total dose of gonadotropin, endometrial thickness, maternal age, the number of previous cycle was statistically significant between groups ( $p < 0.05$ ). In the logistic regression model, age and the number of previous Cycle were negatively associated with pregnancy outcome, while menstrual duration, endometrial thickness, embryo quality and the number of embryos transferred was positively associated with pregnancy outcome. The AUC for the fitted logistic model was 0.681 (95% CI 0.653-0.709) that shows good predictive performance. The calibration plot of the prediction model for pregnancy after ICSI shows that predictive performance of the model is acceptable.

**Conclusion:** Our results indicate that ICSI in an effective option in couples with different cause of infertility. These variables were integrated into a statistical model to allow the prediction of the chance of pregnancy rate in subsequent ICSI cycles.

**Key words:** ICSI, Pregnancy rate, Cause of infertility, Predict.

#### P-110

### Neuroprotective effects of mesenchymal stem cell transplantation in animal model of cerebellar degeneration

Edalatmanesh MA<sup>1</sup>, Bahrami AR<sup>2</sup>.

1. Department of Physiology, Sciences and Research Branch, Islamic Azad University, Fars, Iran.
2. Department of Biology, Faculty of Sciences, Ferdowsi

University of Mashhad, Mashhad, Iran.

Email: ar-bahrami@um.ac.ir

**Introduction:** The cerebellum has been considered a key structure for the processes involved in sensorimotor integration ultimately leading to motor planning and execution of coordinated movement. Thus, motor deficits and behavioral changes can be associated with cerebellar degeneration.

**Materials and Methods:** Here, the chemical neurotoxin pyridine-2, 3-dicarboxylic acid (quinolinic acid, QA) used to create partially cerebellar Stereotaxically administration of QA (0.2 mmol) in the right cerebellar hemisphere (folia VI) caused noticeable motor disturbance in all treated animals. Forty-eight hours after causing lesion, rat bone marrow-derived mesenchymal stem cells (MSCs) were transplanted into damaged cerebellar hemisphere. We investigated the role of MSC transplantation in forms of motor and non-motor learning that involves the cerebellum and its neuroprotective effects in Purkinje cells loss.

**Results:** CM-Dil labeling showed that the transplanted MSCs survived and migrated in the cerebellum 6 weeks after transplantation. The MSC-transplanted group showed markedly improved functional performance on the rotating rod test ( $p \leq 0.0001$ ) and beam walking test ( $p \leq 0.0001$ ) during 6 weeks compared with the controls. For non-motor learning, we used passive avoidance learning test in 3 weeks after transplantation. The results showed that MSC transplantation prevented the development of memory deficit caused by cerebellar degeneration ( $p \leq 0.001$ ). Stereological analysis in 6 weeks after transplantation showed that QA significantly decreases Purkinje cells in vehicle-treated rats and MSC transplantation is neuroprotective and decreases Purkinje cell loss in MSC-treated rats ( $p \leq 0.0001$ ).

**Conclusion:** The results indicate that transplantation of MSCs can significantly reduce the behavioral and neuroanatomical abnormalities of these animals during 6 weeks after engraftment. According to results of this assay, cell therapy by means of bone marrow-derived adult stem cells promises for treatment of cerebellar diseases.

**Key words:** Cerebellar degeneration, Mesenchymal stem cell, Quinolinic acid.

#### P-111

### Differentiation potential of male mouse adipose tissue derived-mesenchymal stem cells into germ cells

Hosseinzadeh Shirzeily M, Pاسبakhsh P, Mehrannia K, Amidi F, Sobhani AGh.

Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Email: sobhanian@tums.ac.ir

**Introduction:** Recent publications regarding to differentiation of stem cells to germ cells have motivated researchers to make new approaches to

infertility. In vitro production of germ cells not only provides new approaches to infertility, but also improves understanding differentiation process of male and female germ cells. Because using embryonic stem cells for this purpose has been associated with tumorigenesis and also ethical criticisms, the mentioned cells were suggested to be replaced with some adult multipotent stem cells. The aim was to find appropriate non invasive source replacement for embryonic stem cells in this study we designed to evaluate differential potentials of adipocyte derived stem cells (ADMSCs) to germ like cells.

**Materials and Methods:** To find differentiation capability, after providing purified ADMSCs differentiation to osteoblast and adipocyte was confirmed by using appropriate culture medium. Superficial markers for mesenchymal stem cells (expression of CD90 and CD73 and non-expression of CD45 and VEGFR2) were investigated by flowcytometry to confirm mesenchymal lineage production. The cells were differentiated to germ cells in mediums containing BMP4 for 5. To evaluate germ cells characteristic markers (MVH, DAZL, STRA8, SCP3) flowcytometry, imunoflorescence and RT-PCR were used.

**Results:** Presentation of stem cell superficial markers (CD90, CD73) and absence of endothelial and blood cell markers (VEGFR2, CD45) were confirmative for mesenchymal origination of these cells. The cells were able to differentiate into osteoblast and adipocyte cells. This fact was representative for multipotential entity of the examined cells. The flowcytometry, imunoflorescence and RT-PCR results showed remarkable expression of germ cells characteristic markers (MVH, DAZL, STRA8, SCP3).

**Conclusion:** By this study, it was found that germ cell markers were expressed in ADMSCs.

**Key words:** Mesenchymal stem cells, Infertility, Germ cells, BMP4, Retinoic acid.

## P-112

### Estrogen receptor $\alpha$ and pinopodes expression analysis at the time of embryo implantation in women with unexplained infertility

Keikha N<sup>1</sup>, Dorostghoal M<sup>1</sup>, Ghaffari HA<sup>2</sup>, Maramazi F<sup>3</sup>.

1. Deptment of Biology, Faculty of Sciences, Shahid Chamran University, Ahvaz, Iran.
2. Deptment of Hematology-Oncology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Obstetrics and Gynecology, Jundishapour University of Medical Sciences, Ahvaz, Iran.

Email: n\_keikha@yahoo.com

**Introduction:** Although several molecules and markers have been proposed to be involved in successful implantation, no marker has been identified to date that is specific and sensitive in identification of receptive endometrium and predicting implantation.

**Materials and Methods:** The presence of developed pinopodes and reduced expression of estrogen receptor

$\alpha$  were reported during the implantation window. The aim of present study was to assess the expression of estrogen receptor  $\alpha$  and pinopodes in endometrium during the mid- Endometrial biopsies were collected from women with normal fertility (n=10) and unexplained infertility (n=27) at peri-implantation periods (LH+7 to LH+8). During the window of implantation in the menstrual cycle formation of pinopodes were analyzed via scanning electron microscopy and expression level of estrogen receptor  $\alpha$  gene was measured with real-time PCR.

**Results:** Our study showed a normal expression of pinopodes during the window of implantation in all biopsies although few pinopodes were present on the endometrial surface in infertile women. Significant increase was seen in estrogen receptor  $\alpha$  expression in patients with unexplained infertility than in the fertile control.

**Conclusion:** Our results provide new information on functional significance of estrogen receptor  $\alpha$  in the initiation and maintenance of the window of receptivity and embryo implantation.

**Key words:** Unexplained Infertility, Implantation, Estrogen receptor  $\alpha$ , Pinopodes.

## P-113

### The effect of hydrostatic pressure on parthenogenetic activation of mouse oocytes in presence of different concentration of extracellular calcium and calcium ionophore and cytochalasin B

Bashiri Z, Azadbakht M, Zeinali H, Goodarzashti A.

Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran.

Email: zbashiri88@gmail.com

**Background:** The artificial activation of eggs induces the completion of oocyte meiosis and is linked to many important problems including that of nuclear transfer. Hydrostatic pressure can act as a mechanical stimulator that rearranges egg contents. Calcium ionophore A23187 is known to elevate intracellular calcium levels in the cytoplasm of oocytes through the influx of calcium from extracellular spaces. In this study, we investigated the effect of hydrostatic pressure on parthenogenetic activation of mouse oocytes in presence of different concentration of calcium and calcium ionophore and cytochalasin B.

**Materials and Methods:** 6 to 8-week-old female NMRI mouse were superovulated by an injection of 10 IU of PMSG, followed by 10 IU HCG 48 hours later. Metaphase II oocytes were collected from oviduct 14 hr after HCG injection. Oocytes transferred to T6 medium supplemented with different concentration of calcium 0, 1.7, 3.4 mM (treatments I, II, III) and 5 $\mu$ M of CI for 5 min. They were divided to experiment and control groups. In experiment group, oocytes subjected to 20 mmHg pressure for 20 min. Oocytes without exposure to pressure were considered as control. Oocytes from two groups were transferred to T6 medium

supplemented with different concentration of calcium and 5µg/ml CB for 4 hours. Oocytes were cultured for 72 hours and embryo development was assessed.

**Results:** In experiment and control groups treatments, II, the percentage of cleavage was 20/42%, 82/78%, 51/6%, 11/36%, 44.27%, 61.35%, respectively. Cleavage rate in experiment group was higher than control group ( $p<0/05$ ). The highest cleavage rate associated with treatment II which were significantly different with treatments, and control ( $p<0/05$ ).

**Conclusion:** Exposure of oocytes to HP, followed by exposure to CI could improve embryonic development and by effecting on calcium channels, leading to increase rate of cleavage in the mouse oocyte, probably.

**Key words:** Parthenogenetic activation, Hydrostatic pressure, Calcium ionophore, Cytochalasin B, Extracellular calcium.

#### P-114

##### Expression of $\beta 3$ Integrin and calcitonin in the peri-implantation period in women with unexplained infertility

Mirani M<sup>1</sup>, Dorostghoal M<sup>1</sup>, Ghaffari HA<sup>2</sup>, Sahbazian N<sup>3</sup>.

1. Deptment of Biology, Faculty of Sciences, Shahid Chamran University, Ahvaz, Iran.
2. Deptment of Hematology-Oncology, Faculty of Medicine, Tehran University, Tehran, Iran.
3. Department of Obstetrics and Gynecology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Email: mmirani65@yahoo.com

**Introduction:** Embryo implantation into the uterus is a critical step in the establishment of pregnancy and failure of this process is a major cause of infertility in women. Different kinds of molecules mediate the interaction between endometrium and embryo. Expression of beta3 Integrin and calcitonin is induced in the human endometrial epithelium specifically during the midsecretory phase of the menstrual cycle, which closely overlaps with the putative window of implantation. Present study was undertaken to evaluate the endometrial calcitonin gene expression in the midsecretory phase of the menstrual cycle in women with normal fertility and unexplained infertility.

**Materials and Methods:** LH timed endometrial biopsies (LH+7 to LH+8) were collected from women with normal fertility (n=10) and unexplained infertility (n=27). Expression level of calcitonin gene in endometrium was measured during the window of implantation in the menstrual cycle, through its mRNA level measurement, with real-time PCR.

**Results:** The levels of endometrial  $\beta 3$  Integrin and calcitonin expression were significantly lower in patients with unexplained infertility than in the fertile control.

**Conclusion:** The present findings suggested that altered expression of  $\beta 3$  Integrin and calcitonin in endometrial cells during the window of implantation may be one of the potential molecular mechanisms of infertility in infertile patients.

**Key words:** Unexplained Infertility, Implantation,  $\beta 3$  Integrin, Calcitonin.

#### P-115

##### Cloning and expression of recombinant human follicle stimulating hormone (hFSH) in CHO cells

Khoshbakht M<sup>1</sup>, Gourabi H<sup>2</sup>, Amiri Yekta A<sup>2</sup>, Fatemi N<sup>2</sup>, Nabiyuni M<sup>1</sup>, Bahraminejad E<sup>2</sup>, Sanati MH<sup>2,3</sup>.

1. Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
3. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

Email: mhsanati@yahoo.com

**Introduction:** Human follicle stimulating hormone (hFSH) belongs to a family of pituitary glycoprotein hormones. This hormone family consists of two  $\alpha$  and  $\beta$  glycosylated subunits. The human FSH plays an important role in oocyte development and maturation in females. In males, the hormone contributes in the regulation of spermatogenesis. The purpose of this study was cloned into Chinese hamster ovary (CHO) cells and expression of recombinant hFSH was studied in the CHO cells.

**Materials and Methods:** In this regard, the ORF region of alpha and beta subunits of hFSH gene was amplified by PCR using specific primers designed (351 and 390 bp respectively) and were cloned into pTZ57R/T vector. Then, the recombinant vectors containing alpha and beta subunits were digested with Sall, BshTI and XhoI, BglII respectively. The subunits were isolated and inserted into pVITRO-Neo-Msc shuttle vector. Designed gene construct was used for introducing into CHO-C111 cell line. Cloning was confirmed by colony PCR.

**Results:** Furthermore, the expression of hFSH gene (alpha and beta subunits) was detected by SDS-PAGE and western blotting techniques in transfected CHO cells.

**Conclusion:** Our results show that the gene construct entailing human FSH sequence can be expressed into CHO-C111 cell line successfully. As a result, production of recombinant protein in the CHO cell is a reliable method for producing of therapeutic human FSH.

**Key words:** Pituitary glycoprotein, Follicle stimulating hormone (FSH), Chinese hamster ovary (CHO), Recombinant.

#### P-116

##### Characteristics of follicular fluid effect with increasing concentration of 50% in hyperactivated human sperm with impaired motility

Naeimi N<sup>1</sup>, Shams lahijani M<sup>2</sup>.

1. Department of Biology, Faculty of Science, Sistan and Baluchestan University, Zahedan, Iran.
2. Department of Biology, Faculty of Science, Sahid Beheshti University, Tehran, Iran.

Email: nasim27bio@yahoo.com

**Introduction:** Infertility treatment is one of the important goals of researchers in the present age. Deficiency in the sperm motility is a problem that can cause infertility in men. Using a natural substance (non synthetic) as FF (Follicular fluid), that can prepare the appropriate conditions in females' genital environment in the laboratory, could be a suitable option, for low cost and easy treatment.

**Materials and Methods:** After studies microscopic and macroscopic on sperm samples (Tehran Navid Infertility Institute), samples were normallized according to WHO and only weak or absent hyperactive motility (grade A) were selected for this experiment. The follicular of fluid when evacuation follicles after centrifuge and filtration of samples were collected and homogenized. Then in 3experimental groups of 55 samples with condition (pre-incubation and without pre-incubation), time (1 and 3 h) and Various doses FF (0%, 25%, 50% and 75%) for study of changes motion hyperactive were evaluated.

**Results:** Progesterone that presents in follicular fluid affects on sperms' acceptance capacity via affecting on calcium channels and gives it a rapid motion as hyperactivation. In this study, after confirmation of the increasing effect of FF in different dosages, on the sperm motion, a significant difference ( $p<0.05$ ) showed the stronger effect of 50% FF for 3 hours in comparison to other concentrations. This solution with mentioned concentration was added in a pre- incubations ordinary issue into sperms with hyperactive deficiency motions. The significant difference between two groups caused the selection of using mentioned. Concentration in ordinary issue (without pre-incubation period) as a preferred method.

**Conclusion:** Therefore, with regarding the available devices in an infertility lab and its' circumstances, it's possible to use FF as a natural substance and less invasive with mentioned conditions in an IUI (intra utrine insemination) to hyperactive impaired motility sperms, to achieve a successful fertilization.

**Key words:** Follicular fluid, Progesterone, Hyperactive motility, IUI.

## P-117

### Identification of site morphine action in pregnant wistar rat placenta tissue: A C<sup>14</sup>-morphine study

**Kazemi M<sup>1</sup>, Sahraei H<sup>1</sup>, Dehghani L<sup>2</sup>, Salehy M<sup>3</sup>, Tekie E<sup>1</sup>.**  
1.Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.  
2.Neuroscience Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran.  
3.Department of Biochemistry, Baqiyatallah University of Medical Sciences, Tehran, Iran.  
Email: mkazemih@yahoo.com

**Introduction:** In previous studies it has been emphasized that the site of morphine action may be either in the embryo or the placenta. In the present study, we attempt to identify the site of morphine action

on the fetal section of Wistar rat placenta by using C<sup>14</sup>-morphine.

**Materials and Methods:** In this study (experimental), female Wistar rats (weights: 170-200 g) were mated with male rats and their coupling times recorded. Experimental groups received daily doses of 0.05 mg/ml of C<sup>14</sup>-morphine in their drinking water. On the 9<sup>th</sup> and 14<sup>th</sup> embryonic days, the pregnant rats were anesthetized and the placenta and uterus surgically removed. Placentas were fixed in 10% formalin for two weeks, then processed, sectioned in 5  $\mu$ m and 25  $\mu$ m thicknesses, and fixed on glass slides for further evaluation. The 25  $\mu$ m sections were delivered to black and white film for three days. Films were processed and evaluated with a digital inverse microscope for possible radiological impression. The 5  $\mu$ m sections were processed for hematoxylin and eosin (H&E) staining, and evaluated by light microscope and MOTIC software.

**Results:** Our results indicated that the site of action of C<sup>14</sup>-morphine was possibly located on the blood plexus of the fetal portion of the placenta. In addition, oral morphine consumption was shown to inhibit fetal and maternal placental development in the experimental groups.

**Conclusion:** We conclude that morphine's effectiveness on the reduction of embryo growth and development may be via its effects on the blood plexus of the fetal section of the placenta. In previous studies it has been emphasized that the site of morphine action may be either in the embryo or the placenta.

**Key words:** Placenta Development, C<sup>14</sup>-Morphine, Rat.

## P-118

### Time-Dependent Effect of Oral Morphine Consumption on the Development of Cytotrophoblast and Syncytiotrophoblast Cells of the Placental Layers during the three Different Periods of Pregnancy in Wistar Rats

**Kazemi M<sup>1</sup>, Sahraei H<sup>1</sup>, Dehghani L<sup>2</sup>, Tekie E<sup>1</sup>, Salehy M<sup>3</sup>.**  
1.Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.  
2.Neuroscience Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran.  
3.Department of Biochemistry, Baqiyatallah University of Medical Sciences, Tehran, Iran.  
Email: mkazemih@yahoo.com

**Introduction:** Morphine consumption during pregnancy causes deteriorates effects on embryos development. The present study investigates the time-dependent effect of oral morphine abuse on cytotrophoblast and syncytiotrophoblast cells layers of placenta development on days 9, 10, 14 of pregnancy in Wistar rats.

**Material and Methods:** Female Wistar rats (W: 170-200 g) have been used in the present study. Experimental groups received morphine (0.05 mg/ml of drinking water) after one night coupling with male rats for mating. On 9<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup> days of pregnancy,

pregnant animals were killed with chloroform and placentas were removed surgically and fixed in 10% formalin. The fixed placentas were processed and stained by Hematoxylin and Eosin method and evaluated for their development. The cells of the placentas layers were calculated by light microscope, MOTIC and SPSS software.

**Results:** The studies demonstrated that the maternal thickness with time-dependant manner increased significantly in comparison with control groups and this change was even more remarkable on day 9-10 of the pregnancy. In addition, the number of cytotrophoblast and syncytiotrophoblast cells in both maternal and fetal parts on the 9<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> days of placentas increased in the experimental groups.

**Conclusion:** The results showed that not only morphine increases the number of trophoblast cell division, but also its destructive impacts are time-dependent.

**Key words:** Development, Placenta, Cytotrophoblast, Syncytiotrophoblast, Morphine, Rat.

## P-119

### The effect of ART on gynecological cancer: Report of our experiences and literature review

Rouhi M<sup>1</sup>, Karimi-Zarchi M<sup>2</sup>, Abdolahi AH<sup>2</sup>.

1.Faculty of Medical Sciences, Islamic Azad University, Yazd Branch, Yazd, Iran.

2.Department of Obstetrics and Gynecology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Email: m.rouhi92@gmail.com

**Introduction:** Infertility is being as an important and common problem in couples that needs to assist reproductive technology (ART) or the other drug therapy. Infertility has been known as a risk factor for ovarian cancer, breast cancer and endometrial cancer but there is a question about the relationship of these cancers to infertility itself or drugs and methods of infertility treatment.

**Materials and Methods:** We evaluated all of risk factors in patients with breast cancer, ovarian cancer and endometrial cancers who referred to Gynecological

Oncology Clinic in Shahid Sadoughi Hospital within 2002-2012. In a retrograde study, we investigated the history of primary infertility and ART before diagnosis of cancers in patients.

**Results:** We registered 92 patients with endometrial cancer, 84 patients with advanced epithelial ovarian cancer and 113 patients with breast cancer. There was infertility history in 39.1% of endometrial cancer who were obese (BMI>30) and 18.8% of patients with normal body mass index (BMI=25-29). ART was founded in 7.3% of all patients with endometrial cancer. Also in patients with epithelial ovarian cancer, female infertility has been diagnosed in 28.4% and ART in 14.1%. Clomiphene therapy with or without HCG and HMG was the most common drugs which were used for patients with ovarian cancer. In all patients with breast cancer, there was infertility in 16.5% and ART was diagnosed in 7.3%.

**Conclusion:** Although infertility was diagnosed as an important and fairly common risk factor in endometrial, ovarian and breast cancer, but some other factors are more important. Age, body mass index and cause of infertility are also important. Finding the association of ART to gynecological cancers need some other long cohort studies which follow the infertile women who get the ART or drug therapy for over 15-20 years. The other studies in this field cannot answer to our question about increasing gynecological cancer due to ART. We think BMI and age are co-factors to cancers which should added to infertility or ART. We had better discuss this relationship to the partners and have a multidisciplinary management for obese infertile women who had had polycystic ovarian syndrome or age more than 35 years. Breast cancer screening should be investigate in infertile women after 35 years because breast is the most common site of primary cancers which send metastasis to ovaries.

**Key words:** Assisted reproductive technologies, Infertility, gynecological cancers, Ovarian cancer, Endometrial cancers, Breast cancers, Polycystic ovarian syndrome, Age, Body mass index.



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