Award Winners

First winners *(Alphabetic order)*

A-1a
Artificial seminal fluid preserves human sperm quality in vitrification program: An electron microscopy study

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Introduction: This study compared the effects of three different media on human sperm parameters, and ultrastructure of spermatozoa using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after vitirification. These media were artificial seminal fluid (ASF), seminal fluid (SF) and human tubal fluid (HTF)-sucrose.
Materials and Methods: 30 normal ejaculates were processed with swim-up technique and sperm suspensions were divided in four aliquots: 1) fresh sample (control); 2) vitrification in HTF supplemented with 0.25 mol sucrose, as routine procedure; 3) vitrification with patients' SF; and 4) vitrification in ASF. After warming, sperm parameters of motility, viability and morphology were analyzed using WHO criteria. Also, sperm pellets were fixed in 2.5% gluthardehyde and processed for SEM and TEM observations.
Results: Sperm parameters in all cryo-groups were reduced when compared with control samples ($p<0.0001$). Briefly, sperm grade A motility, viability and normal morphology were significantly higher in ASF than HTF group. After cryopreservation, deep invagination in cytoplasm, mechanically weak point sites, rough membrane surface and looped tails were observed in SEM evaluation. The looped tails were more severe in SF and HTF cryo-groups. In TEM evaluation, acrosome damage, plasma membrane loss, chromatin vacuolation, and disruption of mitochondria arrangement and structure were observed in all cryo-groups. Degradation of cells was also observed, especially in HTF cryo-group.
Conclusion: Vitrification of human spermatozoa with ASF can effectively preserve the quality of motility in comparison with routine procedure. This ASF medium formulated according to SF that is a natural medium for sperm preservation, lacking artificial components, such as sucrose. With this design, any osmotic shock can be deleted before and after freezing. Also, this study confirmed that SF in normal ejaculates can act as cryoprotectants.
Key words: *Artificial seminal fluid, Seminal fluid, Vitrification, Human spermatozoa.*

A-1b
Proteomic profile of human endometrium in normal women compare to polycystic ovarian syndrome patients

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Introduction: Endometrial receptivity seems to be the major limiting factor for the success of pregnancy in polycystic ovarian syndrome (PCOS). PCOS is the most common cause of female infertility affecting approximately 5-10% of premenopausal women. The aim was to identify the changes in whole proteins between PCOS and normal endometrium.
Materials and Methods: In this study, for the first time, a 2-DE based proteomic approach coupled with mass spectrometry was used to identify the changes in whole proteins between PCOS and normal endometrium. We analyzed proteome of endometrium during proliferative (n=6) and luteal phases (n=6) from healthy women and PCOS patients (n=6). To validate this investigation western blot and quantitative real time PCR were performed.
Results: About 802±10 protein spots reproducible detected on gels, 170 protein spots showed different intensities between PCOS, proliferative and luteal endometrium. Mass spectrometry analysis detected 70 proteins out of 170 spots. The expression of Annexin A5 (ANXA5), 14-3-3 protein, Serpin A1, Cathepsin D proteins was validated by western blot. In addition, the gene expression profile of these proteins was confirmed by real time Q-PCR. The results obtained in the western blot and real time PCR followed a similar regulation of proteomic analysis.
Conclusion: This study provides the first insight into the global protein expression in the endometrium of PCOS patients in compare to normal women which affect endometrial receptivity. Mass spectrometry analysis of differentially expressed proteins between PCOS and normal endometrium resulted in identification of 70 proteins involved in cellular metabolism, oxidative stress, apoptosis and

immunological process. Each of this process absolutely demonstrates an important role in fecundity and fecund ability. So the present study may reveal the cause of various endometrial aberrations in women with PCOS. Our investigation also provides novel information on differential expression of several proteins in secretory phase endometrium compared to proliferative in healthy fertile women. It will create a basis to establish the functional networks that operate as an inducer of the endometrial receptivity.

Key words: *Endometrium, Proteomics, PCOS, Proliferative phase, Secretory phase.*

A-1c

Activation of Toll-like receptor 3 reduces actin polymerization and adhesion molecule expression in endometrial cells, a potential mechanism for viral-induced implantation failure

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Introduction: Embryonic implantation is a critical event which leads to successful pregnancy. This requires communication between the endometrium (mother) and the embryo. It is well-documented that the presence of an infection at the time of implantation can lead to implantation failure. The female reproductive tract recognizes invading microorganisms through the innate pathogen recognition receptors (PRRs) such as the Toll-like receptors (TLRs). To date, 10 members of TLR family have been recognized in human (TLR1 to 10). Our earlier results have demonstrated that the stimulation of TLR 2/6 and 5 in the maternal tract can reduce implantation chances *in vivo* and *in vitro*. In the current investigation, we determined whether the activation of TLR 3 could affect the binding of trophoblast cells to endometrial cells. We also assessed if TLR 3 activation could affect actin polymerization or the expression of adhesion molecules such as β3 and CD98 in endometrial cells, since these changes could represent the molecular mechanism responsible for TLR 3 suppression of trophoblast cells adhesion to endometrial cells.

Materials and Methods: An in vitro assay was developed using RL95-2 (an endometrial cell line) and JAr (a trophoblast cell line) cells. Initially, the percentage of attached JAr spheroids to RL95-2 was measured in response to TLR 3 activation. Next, actin polymerization in RL95-2 cells was assessed in response to TLR 2/6, 3 and 5 activation. Phalloidin was used to assess the mean fluorescence intensity of F-actin by flow cytometry or confocal microscopy. Secondly, the influence of TLR 2/6, 3 and 5 activation on the expression of cluster of differentiation 98 (CD98) and β3 integrin was determined. To further understand through which pathways the TLR 3-induced alterations occur, inhibitors were applied for Toll/interleukin-1 receptor domain-containing adaptor inducing interferon-beta (TRIF), myeloid differentiation primary response 88 (MYD88), mitogen-activated protein kinases (MAPK) and nuclear factor (NF-kB) pathways.

Results: We observed that stimulation of TLR 3 in endometrial cells with different concentrations of Poly I:C led to a reduction of the percentage of JAr spheroids attached to endometrial cells in a dose-dependent manner ($p<0.05$). This decrease was consistent in the Poly I:C treated group regardless of the co-incubation time ($p<0.05$). In addition, our results demonstrated that actin polymerization and CD98 expression significantly decreased only in response to TLR 3 activation ($p<0.05$). Activation of endometrial cells with TLR 2/6, 3 and 5 significantly reduced β3 expression ($p<0.05$). These alterations were shown to work via MYD88-MAPK pathways ($p<0.05$).

Conclusion: TLR 3 activation in the female reproductive tract influenced cytoskeletal changes and adhesion molecules expression in RL95-2 cells in vitro, which can be explained as one of the mechanisms of TLR 3-induced inhibition of trophoblast adhesion to the endometrial cells. This is a novel discovery which extends our current knowledge concerning diagnosis and treatment of viral-induced infertility cases.

Key words: *Implantation failure, Toll-Like receptors, Actin polymerization, Cluster of differentiation 98 (CD98), β3 integrin.*

Second winners *(Alphabetic order)*

A-2a

Preimplantation response to genome instability and prenatal status of genome integrity

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Introduction: Preimplantation DNA damage might alter different pathways including apoptosis; cell cycle and DNA repair. Mosaicism is prevalent in preimplantation stage. A decrease in aneuploidy rate following a prolonged co-culture of human blastocysts has been reported. Differentiation is known as the barrier for elimination of mosaicism; however some mosaicisms could be compatible with live birth.

Materials and Methods: 1) Surplus day-4 embryos of preimplantation genetic screening (PGS) candidates were classified into two groups, with and without signs

of DNA damage, to compare expression of 84 DNA damage signaling pathways genes using PCR array. 2) We used FISH to reanalyze surplus blastocysts following day 3 PGS. 3) Forty four tissues of two apparently normal fetuses were studied using microarray for mosaicism analysis following therapeutic abortion due to maternal indications. Reciprocal aberrations validated by qPCR.

Results: 1) Five of the 84 studied genes (*MSH3*, *XRCC1*, *RAD50*, *LIG1* and *CDK7*) overexpressed in embryos with signs of DNA damage. 2) Prolonged culture was not efficient to decrease aneuploidy. Mosaicism observed in 86.6% of the blastocysts; frequency of normal cells in day 7 blastocysts was lower than that of day 6. 3) Among explored Copy Number Variations (CNVs) in the tissues of the first and the second fetuses, 67 and 45 CNVs related to 13 and 14 cytogenetic locations were reciprocal, respectively. Some CNVs were limited to one or two tissues while some others were seen in several tissues.

Conclusion: 1) The altered genes are involved in DNA repair, therefore the dominant response to preimplantation DNA damage is DNA repair rather than cell cycle control or apoptosis. 2) Despite activated DNA repair pathways, the widespread abnormality in blastocysts indicates poor performance of aneuploidy correction in preimplantation stage. If apoptosis was dominant response, predictable aneuploidy was lower than what was occurred. 3) Distribution pattern of frequent CNVs indicates preimplantation origin while CNVs with low frequency likely occurred in later stages. Regarding preimplantation origin of some prenatal mosaicisms, high resolution PGS for detection of mosaic embryos in CNV level could be helpful for transfer of healthier embryos.

Key words: *Preimplantation, DNA damage, Gene expression, Prolonged culture, Mosaicism, Prenatal.*

A-2b

New insight into endometriosis pathogenesis, recurrence and treatment approach

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Introduction: Endometriosis can be regarded as a benign metastatic disease. The pathogenesis of endometriosis involves complex mechanisms such as malignant-like mechanisms. *HOX* genes are necessary for endometrial growth, differentiation and implantation and have a critical role in cancers and endometriosis. To determine cause of endometriosis recurrency, we investigated expression of 84 *HOX* genes in endometriosis compare to eutopic tissues and normal endometrium.

Materials and Methods: Samples obtained from 15 patients with endometriosis and 15 controls without endometriosis were collected. All participates were at reproductive age with normal menstrual cycles, where the same patients provided both eutopic and ectopic endometrium (endometriomas) and control samples were surgically checked for the absence of endometriosis. The expression profile of 84 genes of *HOX* family related to various aspect of cell proliferation was investigated using a qRT-PCR array. Informed consent was obtained from patients. All measurements were performed in triplicates on independent biological replicates.

Results: Expression of the 54/ 84 studied genes showed significant difference between groups. Our data showed significant over-expression of some genes which are involved in regulation of development (*SHOX*, *SHOX2*), prevention of apoptosis and promotion of cell proliferation (*DLX* 3, 4, 5 and 6), regulation of collagen expression (*MKX*) as well as *HOXC* and *HOXD* cluster in ectopic versus euotopic and control tissues, which indicated invasive property of endometriosis. Down-regulation of *HOXA* and *HOXB* cluster and some genes involved in apoptosis (*MSX1*, *MSX2*), also was observed in ectopic versus euotopic tissue.

Conclusion: Aberration in *HOX* genes expression especially genes which are involved in various aspect of cancer, including cell proliferation, invasiveness and progression, may lead to recurrent of endometriosis. So the surgeons should remove any visible implants and scar tissue with its margins, if retain any cells in the site of ectopic tissues, its can proliferate and invade. Finally it is lead to formation of a new lesion and actually recurrent of disease.

Key words: *HOX genes, Endometriosis, Recurrence.*

A-2c

Comprehensive chromosome screening of single sperm using a whole genome sequencing technique

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Introduction: About 40% of infertile men have normal semen parameters. The failure of conventional semen analysis to identify abnormalities leads to problems for the accurate characterization of infertility and, as a consequence, difficulties counseling patients and selecting optimal treatments. One factor, invisible to standard sperm assessment, which contributes to male infertility, is aneuploidy. The assessment of chromosomal aneuploidy in sperm has been challenging due to the highly condensed nature of the DNA. Fluorescent in situ hybridization (FISH) is the most common method for assessing sperm aneuploidy, but

only analyses a handful of chromosomes (typically just five). This study aimed to develop a novel protocol permitting comprehensive analysis of chromosomes in individual sperm.

Materials and Methods: 30 single sperm from a male with normal semen parameters were isolated by micromanipulation and the whole genome amplified using multiple displacement amplification (MDA). The MDA products were subjected to Next-Generation Sequencing (NGS) using an Ion Personal Genome Machine. The proportion of DNA fragments attributable to each chromosome was assessed. Excessive/deficient numbers of DNA fragments from individual chromosomes were indicative of aneuploidies. Additionally, DNA fingerprinting was applied to each MDA product, revealing any instances where two sperm had inadvertently been placed in the same sample tube.

Results: NGS permitted sequencing of the genome of each sperm to an average depth of 0.1X. All of the samples considered for NGS were confirmed to be single sperm. Analysis of the data revealed 3 chromosomally abnormal sperm. Abnormalities included +12 and +17, aneuploidies that would not be detected using standard FISH.

Conclusion: This study demonstrates the technical feasibility of NGS applied to single sperm and its advantage in providing a comprehensive chromosome assessment. The method is immediately applicable for research purposes, but is currently expensive. However, the rapidly declining costs of NGS mean that future applicability in a clinical context is likely.

Key words: *Sperm, Aneuploidy, NGS.*

Third winners*(Alphabetic order)*

A-3a

Efficacy of transvaginal perfusion of granulocyte colony stimulating factor on recurrent implantation failure: Randomized control trial

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Introduction: Repeated implantation failure (RIF) is due to poor quality of embryo, endometrium, uterine and fallopian tube. Also perinatal and immunologic factors can be noted. RIF means failure of 2 IVF cycle in patients with more than 10 high quality embryos. Granulocyte colony stimulating factor (GCSF) is a glycoprotein that stimulates cytokine growth factor and induced immune system. The aim of this study was to evaluate GCSF ability to improve pregnancy rate in women with repeated implantation failure.

Materials and Methods: This was a randomized control trial which conducted in Yazd Research and Clinical Center for Infertility, 2014-2015. Women with history of RIF and under 40 years old were included. Participants with GCSF contraindication, endometriosis and sever male factor were excluded. Totally 90 eligible women were randomly allocated in two groups. All of participants received antagonist protocol. Then 30 ml (300 mg/ml) GCSF was administered in intervention group by intrauterine infusion. Pregnancy outcomes were assessed based on chemical and clinical pregnancy.

Results: Totally 90 patients were included. The mean age of participants was 31.95±4.71 years old. There were no differences in ART and demographic characteristics of two groups ($p>0.05$). The pregnancy outcome in GCSF group was improved significantly ($p=0.043$).

Conclusion: In this RCT we could detect a significant treatment effect of GCSF on pregnancy rates. GCSF can improve pregnancy outcome in patients with RIF.

Key words: *GCSF, Pregnancy rate, Repeated implantation failure.*

A-3b

Transvaginal perfusion of granulocyte colony stimulating factor for infertile women with thin endometrium in frozen ET program: A non-randomized clinical trial

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Introduction: We often see patients with a thin endometrium in ART cycles, in spite of standard and adjuvant treatments. Improving endometrial growth in patients with a thin endometrium is very difficult. Without adequate endometrial thickness these patients, likely, would not have reached embryo transfer. We planned this study to investigate the efficacy of intrauterine granulocyte colony stimulating factor (GCSF) perfusion in improving endometrium, and possibly pregnancy rates in frozen-thawed embryo transfer cycles.

Materials and Methods: This is a non-randomized intervention clinical trial. Among 68 infertile patients with thin endometrium (-7 mm) at the 12^{th}-13^{th} cycle day, 34 patients received GCSF (300 microgram/1mL) to improve endometrial thickness by direct administration by slow intrauterine infusion using IUI catheter. If the endometrium had not reached at least to 7-mm within 48-72 hr, a second infusion was given. Endometrial thickness was assessed by serial vaginal ultrasound at the most expanded area of the endometrial stripe.

Results: The cycle was cancelled in the patients with thin endometrium (endometrial thickness below 7mm) until 19th cycle day ultimately. The cycle cancelation rate owing to thin endometrium was similar in GCSF

group (15.20%), followed by (15.20%) in the control group (p=1.00). The endometrial growth was not different within 2 groups, an improvement was shown between controlled and GCSF co-treated groups, with chemical (39.30% vs. 14.30%) and clinical pregnancy rates (32.10% vs. 12.00%) although the differences were not significant.

Conclusion: Our study fails to demonstrate that GCSF has the potential to improve endometrial thickness but it shows that GCSF has the potential to improve chemical and clinical pregnancy rate of the infertile women with thin endometrium in frozen-thawed embryo transfer cycle.

Key words: *Thin endometrium, Granulocyte colony-stimulating factor, Frozen embryo transfer, Pregnancy rate, Implantation.*

A-3c

Efficacy of motile sperm organelle morphology examination (MSOME) and sperm head vacuoles evaluation in conventional IVF versus ICSI cycles

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Introduction: The impact of MSOME criteria on ICSI outcomes is a controversial issue in the literature. There are rare studies regarding the association between anteroposterior sperm head size, cytoplasmic droplet, head shape and conventional sperm parameters. To the best of our knowledge, the prevalence of sperm deformities using MSOME has not been reported in conventional IVF cycles.

Materials and Methods: This is a prospective analysis of MSOME parameters in IVF (n=31) and ICSI cycles (n=35) performed from 2013 to 2014. MSOME parameters were evaluated as follows: vacuole: none, small, medium, large and mix; head size: normal, small and large; cytoplasmic droplet; head shape and acrosome normality. We compared the association between MSOME and conventional sperm parameters, early embryo development and pregnancy outcomes.

Results: In IVF group, the rate of large nuclear vacuole (LNV) was significantly lower in successful pregnancies compared to non-pregnant patients (7.38±4.4 vs. 13.81±9.7, respectively, p=0.045). Conversely, the rate of small nuclear vacuoles (SNVs) showed increased level in positive pregnancies. There was a positive correlation between the rates of non-vacuoles, SNVs and normal sperm shape (p<0.0001 and p=0.003, respectively). A negative correlation was found between the rate of LNVs and normal sperm shape. Moreover there was a positive correlation between progressive motility and normal head size. In ICSI group, we did not observe any association between MSOME criteria and pregnancy outcome. The rate of LNVs and large head sperm size illustrated significantly positive correlation with the percentage of non-progressive motile sperm. Also, the cytoplasmic droplet had negative effect on sperm shape (p=0.04).

Conclusion: The LNV has negative effect on sperm shape normality and pregnancy outcome in IVF cycles. However, there was no any association between MSOME parameters and early embryo development and pregnancy outcome in ICSI cycles.

Key words: *MSOME, IVF, ICSI, Sperm head vacuoles.*

A-3d

Short-term culture of human ecto-cervical epithelial cells for genomic, proteomic and functional studies

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Introduction: Understanding cell physiology is limited by reliance on tumor-derived immortalized cell lines. Primary cell culture models may offer more relevant mechanistic insight into cell physiology but are often difficult to establish and maintain. We sought to develop an optimal method for the isolation and short-term culture of human primary ecto-cervical epithelial cells (HECECs).

Materials and Methods: Fresh ecto-cervical tissues were obtained at hysterectomy and epithelia was isolated and cultured (using MEM D-Valine media to prevent fibroblast proliferation) using three different explants methods: i) tiny fragments of epithelium; ii) dissociated cells cultured after digestion using Collagenase IV and trypsin; and iii) digested tissue clumps. The epithelial phenotype of cultured cells was verified by double immunofluorescence sequential staining to detect cytokeratin, specific antigen for epithelial cells. The expression of oestrogen (ERα, ERβ) and progesterone receptors (mPRα, mPRβ, PRγ and nPRA and nPRB) genes were investigated by RT-PCR. Flow cytometry was employed to detect TLR2 and TLR4, receptor targets for our proposed functional studies of pattern recognition in the human cervix.

Results: Cultures were successfully established using all three methods but cell growth was best from digested tissue clumps which were employed for subsequent experiments. Primary cells were sub-cultured at least two times. Exclusion of fibroblasts from cultures was confirmed by the absence of staining to CD90. We confirmed the expression of all *ER* and *PR* genes, as well as the expression of TLR2, TLR4 in derived HECECs.

Conclusion: HECECs cultured from explants of digested tissue clumps, employing our protocol, yield

enough pure epithelial cell population, uncontaminated by stromal fibroblasts, which are suitable for molecular investigations involving a small number of passages.

Key words: *Cell culture, Epithelial cells, Ecto-cervix, TLR.*

A-3e

The viability rate of ovine spermatogonial stem cells after cryopreservation in different concentrations of FBS

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Introduction: Spermatogonial Stem Cells (SSCs) have the main role in spermatogenesis process. This type of stem cells possess the ability of differentiating into three germ layer lineages, so could be used for treating some kinds of infertility disorders in male patients. Also in animal reproductive technologies such as artificial insemination, transgenesis and grafting, SSCs are important supplement. For this purpose, testicular cells can be kept for long or short time. One of keeping procedures is cryopreservation, that many kinds of cryoprotectant agents are available. The higher viability rate of frozen-thawed cells, the better cryopreservation agent is.

Materials and Methods: Testicular cells were extracted from six 2-month old lambs by testicular biopsy (TESE) and bi-step enzymatic digestion. For spermatogonial stem cells and Sertoli cells quiddity confirmation, immunocytochemical analysis was used. Anti Oct-4 and anti vimentin were immunocytochemical markers. The suspended cells were collected and cultured for 12 days. The cells were frozen in two cryopreservation groups for one month in -196 degrees centigrade. The first cryopreservation group included 50% fetal bovine serum (FBS) while the second group had 70% FBS. In all groups 10% Dimethyl Sulfoxide (DMSO) was used as a cryoprotectant agent. The viability of each two types of frozen-thawed cells was assessed with trypan blue staining procedure after 1 month.

Results: There is a direct relationship between the increasing FBS concentration in cryopreservation media and the viability rate of frozen-thawed testicular cells. The viability rate of testicular cells was 64.12% for first cryopreservation group (contained 50% FBS), 67.03% for the second one (contained 70% FBS) and 89.19% for control group.

Conclusion: Based on literature, FBS containing cryopreservation media, have better cyroprotective function for SSCs cryopreserving compared to other well-known cryoprotectant agents.

Key words: *Sheep, Spermatogonial stem cell, Cryopreservation, Fetal bovine serum.*

Fourth winners *(Alphabetic order)*

A-4a

Association between nuclear receptors of estrogen and progesterone with adiponectin receptors in granulosa cells of patients with poly cystic ovary syndrome

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Introduction: The poly cystic ovary syndrome (PCOS), one of the most common endocrine disorders in reproductive age women, is associated with obesity and insulin resistance predisposing to diabetes mellitus type 2 and atherosclerosis. Adiponectin is a recently discovered adipocytokine with insulin –sensitizing and putative anti atherosclerotic properties. Several studies have illustrated that adiponectin can regulate granulosa cell stroidogenesis and the expression of genes associated with ovulation. Therefore, the aim of this study was to investigate a relationship between gene expression of estrogen and progesterone nuclear receptors and adiponectin receptors in granulosa cells (GCs) of PCOS women compared to women with normal cycling ovaries in order to achieve a better understanding of ovarian steroid status in patients with PCOS.

Materials and Methods: In this prospective study, 40 patients with PCOS and 40 women with normal ovulatory function who underwent IVF for treatment of tubal and/or male infertility were recruited. Follicular fluid was collected from patients and. GCs were isolated from follicular fluid by centrifugation and then were purified with Micro Beads conjugated to monoclonal anti-human CD45 antibodies. RNA was extracted and Reverse transcription was performed. Gene expression of AdipoR1, AdipoR2, estrogen and progesterone receptors was determined by quantitative real time PCR (q-PCR). All statistical procedures were run on SPSS 16. $P \leq 0.05$ was considered significant.

Results: By considering all subjects with and without PCOS undergoing controlled ovarian hyper-stimulation, we observed ERα and ERβ mRNA expression correlated positively with the mRNA

expression of AdipoR1 (r=0.85, p=0.0001 and r=0.92, p=0.0001, respectively) and AdipoR2 (r=0.87, p=0.0001 and r=0.88, p=0.0001, respectively). Estrogen receptor β (ERβ) expression was significantly higher compared to ERα expression in both groups (p<0.002). Moreover, progesterone receptor A (PRA) and PRB were both expressed in human GCs. However, the expression level of nuclear PRB was very low in both groups (p<0.008). There was a significant correlation between progesterone receptors and adiponectin receptors (r=0.8, p=0.0001 and r=0.88, p=0.0001). In our present results, increased ratio of PRA/PRB in women with PCOS has been revealed.

Conclusion: This research provides more evidence about expression profiles of genes involved in metabolism, steroidogenesis and ovulation in PCOS and supports the hypothesis that abnormal hormone activity, by different receptor expressions, may be an important factor in the generation of ovarian disorder.

Key words: *AdipoR1, AdipoR2, Estrogen receptor (ER), Progesterone receptor (PR), Granulosa cell (GC), polycystic ovary syndrome (PCOS).*

A-4b
GDF-9 supplementation improved embryo formation rates in clinical IVM program

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Introduction: GDF-9 is an oocyte-secreted GF which is critical for promotion of in-vitro growth of ovarian follicle and preovulatory cumulus cells (CCs) expansion. Supplementation of GDF-9 in IVM medium may enhance embryo development and fetal viability. The aim was to investigate the effects of GDF9 supplementation in IVM medium for human GV oocytes retrieved from ICSI cycles, on rates of oocyte maturation, fertilization, and subsequent embryo development.

Materials and Methods: Retrieved GV oocytes were divided in 4 groups. In group I, 108 oocytes were cultured in commercial IVM media (control); in group II, 68 oocytes were cultured with CCs; in group III, 66 oocytes were cultured in media supplemented with 200 ng/ml GDF-9; and in group IV, 99 oocytes were cultured with CCs in presence of 200 ng/ml GDF-9 at 37°C, 5% CO_2. Maturation was considered when oocytes excluded 1st polar body. Matured oocytes were screened for ZP birefringence and meiotic spindles (MS) with Polar Aide Microscopy. After ICSI, normal fertilization and cleavage rates were analyzed.

Results: Although, the maturation rate of control group (63.9%) was higher than other groups; but,in the process of fertilization (group I vs. II, p=0.01), up to embryo formation (group I vs. II, p=0.001 and group I vs. III, p=0.05) remarkable reduction was observed in group I. In term of maturation and fertilization rates, there were no significant differences between experimental groups. However, both embryo formation and quality of group III were better than the other groups. Among matured oocytes, the rates of oocytes with spindles in group III were lower than oocytes in group IV (24.6% vs. 45%, p=0.03). Whereas, the percentage of high birefringence (HB) oocytes in group I was higher than group IV (63.8% vs. 38.2%, p=0.005).

Conclusion: Application of exogenous GDF9 during clinical IVM improved embryo development. It seems a promising approach for improving human IVM program.

Key words: *Human oocyte, IVM, GDF-9, Fertilization, Embryo.*

A-4c
Effect of single dose GnRH agonist on pregnancy outcome in frozen-thawed embryo transfer cycles

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Introduction: There is no dout that luteal phase support (LPS) is essential to enhance the reproductive outcome in IVF cycles. In addition to progesterone and human chorionic gonadotropin, several studies have described GnRH agonists as LPS to improve implantation rate, pregnancy rate and live birth rate, whereas other studies showed dissimilar conclusions. All of these studies have been done in fresh IVF cycles. This prospective controlled trial was designed to test this hypothesis in frozen-thawed embryo transfer cycles (FET cycles).

Materials and Methods: In 200 FET cycles, patients were randomized on the day of embryo transfer into group 1 (n=100) to whom a single dose of GnRH agonist (0.1 mg triptorelin) was administered three days after transfer and group 2 (n=100), who did not receive agonist. Both groups received daily vaginal progesterone suppositories (800 mg daily) plus estradiol valerate 6 mg daily. The primary outcome measure was clinical pregnancy rate and the secondary outcome measures were implantation rate, chemical andongoing pregnancy rate and abortion rate.

Results: A total of 200 FET cycles were analysed. Demographic data and embryo quality were comparable between two groups. No statistically significant difference in chemical, clinical, ongoing and abortion rate was observed between the two groups (26% vs. 21%, p=0.4) and (21% vs. 17%, p=0.47).

Conclusion: Administration of a subcutaneous GnRH agonist at the time of implantation does not increase clinical or ongoing pregnancy rate in FET cycles.

Key words: *Frozen-thawed embryo transfer cycles, GnRH agonist, Luteal phase support.*

A-4d
Sperm chromatin condensation, DNA fragmentation and apoptosis in globozoospermic patients

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Introduction: Globozoospermia is a severe form of teratozoospermia with very low incidence in infertile patients that is characterized by round sperm head and lack of acrosome. It's considered as one of the important causes of male infertility which the success rate in assisted reproductive technology (ART) cycles is also low. The goal was to compare the semen parameters and chromatin/DNA integrity as well as apoptosis in ejaculated spermatozoa between globozoospermic and normozoospermic men.
Materials and Methods: In total 57 men were divided into two groups including globozoospermic (n=27) and normozoospermic men as controls (n=30). Semen analysis was performed according to WHO criteria (2010). Sperm chromatin condensation and DNA integrity were assessed using cytochemical tests including: Aniline blue (AB), Toluidine blue (TB), Chromomycin A3 (CMA3) and Sodium dodecyl sulfate (SDS) for chromatin compaction and Acridine orange (AO), Sperm chromatin dispersion (SCD) and TUNEL assays for DNA structure and apoptosis detection.
Results: There were significant differences regarding sperm count, motility and normal morphology between two groups. The percentage of abnormal chromatin packaging/ DNA integrity (using AB, TB, AO and SCD tests) was significantly higher in globozoospermic men compared to normozoospermic samples. The rate of spermatozoa with protamine deficiency (CMA3+) showed an increase in golobozoospermic patients when comparison with controls (63.59±13.29 vs. 24.17±9.5, respectively, $p<0.0001$). it should be noted that in SDS test, we didn't any significant difference between groups. But, the rate of TUNEL positive spermatozoa were significantly increased in globozoospermic cases respect to the controls (14.81±9.91 vs. 5.95± 3.02, respectively, $p<0.0001$). There was no significant correlation between sperm DNA denaturation, DNA fragmentation and apoptosis in globozoospermic men. Our data showed significant correlation between single strand DNA (AO+) and progressive motility ($p=0.036$).
Conclusion: The rate of spermatozoa with abnormal chromatin packaging, DNA damage and apoptosis were significantly higher in globozoospermic samples than normal fertile men. However, the sperm chromatin/ DNA anomalies may be considered as one of the main etiologies of ART failure in these patients.

Key words: *Globozoospermia, Male infertility, Sperm chromatin, DNA integrity.*

A-4e
Does cosmetic micromanipulation of vitrified-warmed preimplantation embryos enhance pregnancy rate?

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Introduction: Cytoplasmic fragmentation in cleaving embryos which is cornerstone of each embryo grading system has been shown to be an important biomarker for implantation potential. Beside fragmentation, another dysmorphism is the presence of coarse granulation in perivitelline space (PVS). In our hypothesis, the cosmetic micromanipulation (CM) is defined as removal of fragments, coarse granules, and attached cumulus cells (CCs) to zona pellucida from the vitrified- warmed embryos before embryo transfer (ET). The objective was to investigate the effect of CM on the subsequent cell division, morphology and pregnancy outcomes of the vitrified-warmed fragmented human embryos.
Materials and Methods: Patients undergoing frozen ET (FET) with similar clinical characteristics were included in this ongoing prospective randomized study. They were divided into three groups of CM, laser assisted zona hatching (LAH) and control. The vitrified-warmed embryos with >10% and <50% fragmentation met inclusion criteria. In CM group, five hours after embryo warming and morphology evaluation, the embryos were subjected to CM after LAH .Whereas; in LAH group warmed embryos were subjected to LAH only. After overnight incubation, cell division and morphology of embryos were evaluated and divided embryos were transferred.
Results: The morphological grade of fragmented embryos improved after the CM. Most of the fragmented embryos did not show a regeneration of fragments after CM during the subsequent development, and a beneficial effect of CM on the development of the embryos was observed. Pregnancy rates in CM, LAH and control groups were 42.8%, 37.5% and 45.4% respectively. There were no statistical significance ($p>0.05$) in pregnancy rates between the groups because of the low number of trials.
Conclusion: CM improved the subsequent development as well as the morphological grades of fragmented embryos. But, our preliminary data showed that this technique in FET cycles neither compromise nor improved pregnancy outcomes in unselected patients. Further controlled trials will determine whether CM can improve pregnancy outcomes in a selected patient population, such as recurrent implantation failure and advanced maternal age.

Key words: *Cosmetic microsurgery, Fragment removal, Pregnancy, Vitrified-warmed embryo.*