Award Winners *(Alphabetic order)*

A-1

ER-mediated actions, even in the absence of AR actions, can induce reproductive features of PCOS

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Background: Hyperandrogenism is a key defining feature of polycystic ovary syndrome (PCOS). Clinical and animal experimental studies have provided strong evidence to support an important role for androgens in driving the development of PCOS. Testosterone (T) and dihydrotestosterone (DHT) are the two bioactive forms of androgens which can act directly through the androgen receptor (AR). Additionally, T, unlike DHT, can also be converted to estradiol and act via the estrogen receptor (ER).

Objective: To determine if ER-mediated actions are involved in the development of different features of PCOS.

Materials and Methods: ARKO mice were generated using Cre/LoxP technology. WT and ARKO prepubertal mice were implanted with a blank, T or DHT implant and examined after 12 weeks.

Results: Both T and DHT induced anovulation in WT mice as there were no corpora lutea (CL) in their ovaries (CL number: WT+blank: 9.5±2.1; WT+DHT: 0±0; WT+T: 0±0, $p<0.01$). In contrast, ARKO mice treated with blank or DHT implants ovulated, but those with T implants still displayed ovulatory disruption (CL number: ARKO+blank: 2.3±0.3; ARKO+DHT: 3.25±0.4; ARKO+T: 0.4±0.4, $p<0.05$). This finding suggests that ER actions even in the absence of AR actions can induce disrupted ovulation. In WT mice, DHT but not T induced metabolic features of PCOS (e.g. body weight (WT+blank: 23.4g±0.5; WT+DHT: 27.1g±0.6; WT+T: 22.5g±0.67, $p<0.01$)), however neither androgen had an effect in ARKO mice (body weight, ARKO+blank: 23.1g±0.4; ARKO+DHT: 24.1g±0.7; ARKO+T: 23.5g±0.4). Indicating the role of AR, but not ER, in the development of metabolic features of PCOS.

Conclusion: These results show a key role for AR signalling in the establishment of reproductive and metabolic traits of PCOS, but also implying that ER-mediated actions may contribute to the development of reproductive features of PCOS. Suggesting that non-hyperandrogenic PCOS patients (phenotype D) may have a different etiology and prognosis from hyperandrogenic-PCOS patients.

Key words: *PCOS, Androgen receptor, Estrogen receptor, ARKO.*

A-2

Creating a sustained release of clomiphene citrate upon administration in nano phosal-based formulation (PBF) and assessment of endometrial receptivity genes by influence of Nano clomiphene citrate

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Background: Stimulation of ovulation by clomiphene citrate can cause side effect on endometrial receptivity. Formulation Nano may be an alternative therapeutic for women with ovulatory disorders.

Objective: The overall objective of this study was to develop an oral sustained release clomiphene citrate by using Phosal-Based Formulation (PBF) and evaluation decreased its side effect on endometrium and compare the expression of endometrial receptivity genes in implantation, in mice stimulated with clomiphene citrate encapsulated in PBF (CC/PBF) and clomiphene citrate free clomiphene citrate.

Materials and Methods: In in vitro study CC loaded PBF was analyzed using zeta sizer, FTIR, TEM. In in vivo study, 18 female mice were randomly divided into 3 groups CC/PBF, CC, normal saline (SS) and daily administered 200µl and injected with 5IU HCG and mated after two days. At day 4.5, pregnant mice were euthanized, and endometrial tissue were extracted for Q-PCR analysis.

Results: The optimized PBF contained Phosal 50PG/glycerol in a 2:8 ratios (w/w) and mean particle size of Nano drug used were 67±9.0 nm, the release of CC from Nano emulsion was slightly faster in the first 24 hours; during this period, 29% of CC was released. After 120 hours 76% of CC was released. LIF, LIFR, HOXA10, HBEGF, EGF mRNA levels were significantly upregulated and MUC1, PGR mRNA levels were significantly downregulated in the CC/PBF treated animals compared with the CC group ($p<0.05$).

Conclusion: Formulation sustained release of clomiphene citrate increased its targeting efficiency and improved the impact of the CC on the serum levels of estradiol and expression of genes involve implantation. A new Phosal-Based Formulation (PBF) was introduced to decrease side effect of clomiphene citrate on endometrium, this drug formulation could react better in implantation and preventing abortion by increasing genes involved in implantation, the in vivo study demonstrated that the PBF in mice has a significantly higher genes

expression involved implantation than that of the systemic formulations.

Key words: *Endometrial receptivity, Clomiphene citrate, PBF, Sustained release, Drug delivery, Ovarian stimulation, OHSS, Nano drug.*

A-3

Association between early embryo morphokinetics plus cumulus cell gene expression and assisted reproduction outcomes in polycystic ovary syndrome women

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Background: It is shown that oocyte cumulus cells are associated with embryo quality and reproductive outcomes in assisted reproductive technology.

Objective: To evaluate a combination of time-lapse morphokinetic parameters and cumulus cell gene expression in polycystic ovary syndrome (PCOS) women for predicting assisted reproductive treatment outcome.

Materials and Methods: A total of 547 embryos from 100 intracytoplasmic sperm injection (ICSI) cycles were evaluated. Fifty women with PCOS and 50 women who were categorized as tubal factor infertility were recruited. Time-lapse records were annotated for time to pronuclear fading (tPNf), time to 2 to 8 cells (t2-t8), reverse cleavage, direct cleavage and also for the presence of multinucleation. Expression levels of three genes involved in mitotic divisions, diaphanous-related formin 2 (DIAPH2), nibrin (NBN) and NIMA-related protein kinase (NEK4), were measured in 100 associated cumulus cell samples using quantitative real-time polymerase chain reaction.

Results: Expression of DIAPH2 and NBN was significantly higher in the embryos of PCOS patients that resulted in implantation, biochemical and clinical pregnancies as well as live birth compared with embryos that were negative for these outcomes ($p<0.01$). However, in the tubal factor group, NBN gene expression was significantly higher in embryos resulting in biochemical pregnancy, clinical pregnancy and live birth ($p<0.01$) only. Multivariate logistic regression analysis showed that tPNf together with DIAPH2 gene expression were independent prognostic factors of clinical pregnancy rate and live birth in both groups.

Conclusion: Some time-lapse embryo parameters may be related to cumulus gene expression and clinical outcome. Furthermore, the expressions of cumulus cell genes involved in mitotic divisions are significantly associated with ICSI outcome using Day 3 embryo transfer.

Key words: *Cumulus cell, Embryo morphokinetics, Gene expression, Polycystic ovary syndrome, Pregnancy outcome.*

A-4

Genotype-phenotype correlation of morphological abnormalities of the sperm flagella (MMAF) in infertile men by Whole exome sequencing

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Background: Multiple morphological abnormalities of the flagella (MMAF), is one of the most severe form of asthenozoospermia and is characterized by the simultaneous presence of five morphological defects of the sperm flagella (absent, short, bent, coiled flagella and flagella of irregular width). Many cases of infertile males are categorized to be idiopathic, indicating that the cause is unknown and the mechanisms responsible for their condition are to be found. To date, a short list of genes was identified which is in sharp contrast with the fact that several hundreds of genes are estimated to be involved in spermatogenesis and male reproduction. However, gene identification is the key to improving knowledge of the pathophysiology of MMAF and opens new perspectives for diagnosis and treatment of infertile patients.

Objective: Further genetic studies are therefore warranted to identify other genes involved in MMAF to better characterize the genetic etiology of the MMAF phenotype and to improve the management of patients diagnosed with flagellar defects.

Materials and Methods: In our study, we analyzed 78 MMAF patients using WES and showed that mutations in DNAH1, CFAP43, CFAP44, CFAP69, WDR66, and FSIP2 are responsible for MMAF syndrome. After Sanger Sequencing verification of all candidate variants including, Relative mRNA expression levels for the selected candidate genes were assessed by qRT-PCR. To characterize the structural and ultrastructural anomalies present in patients" sperm, immunofluorescence was performed on sperm samples from the subjects with a mutation and one control and transmission electron microscopy (TEM) analyses was performed on sperm samples. Most importantly, we investigated the role of some of these novel genes by performing gene invalidation and silencing in two evolutionary distant models sharing an extremely conserved flagellar structure, Trypanosoma and mouse.

Results: Overall, DNAH1, CFAP43, CFAP44, CFAP69, WDR66, and FSIP2 mutations were identified in 45% of the analyzed subjects (35 out of 78 patients) originating from the Middle East, North Africa, and Europe. None of these mutations were reported in control sequence databases. TEM analyses showed a complete disorganization of the fibrous sheet associated with axonemal defects. Immunofluorescence analyses confirmed that the central-pair microtubules and the inner and outer dynein arms of the axoneme were abnormal in the patients carrying these mutations.

Conclusion: Altogether, our results underline the global importance of these 6 genes in the MMAF syndrome and will improve the genetic diagnosis efficiency of infertile MMAF patients. In our study, WES revealed that the aforementioned genes are the main genes involved in MMAF phenotypes. Our work illustrates the efficiency of the combination of WES with original workflow for the validation of the candidate genes that are identified in male infertility due to a MMAF phenotype and exploit the WES data to the benefit of the patients.

Key words: *Male infertility, Genetic diagnosis, Exome sequencing, Teratozoospermia, MMAF.*

A-5
The tissue engineered uterus by 3D printing

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Background: The uterus is a female organ of the reproductive system. 3D printing as a method by which complex and carefully design biological structures of in vivo tissues are simulated is of a great interest in regenerative medicine and tissue engineering in reproductive system. Solved biomaterials that are used, as bio-inks in 3D printing should be biocompatible, degradable, absorbable and cell friendly, are so significant in development of 3D printed uterus construct. In spite the fact that different kinds of synthetic materials as a bio-ink have been applied for uterus 3D printing, due to their insufficiency of fundamental proteins and being poor in mechanical and surface properties, their performance were not acceptable.

Objective: We decided to apply PLA as a proper synthetic material for producing scaffold of tissue engineered uterus to culture endometrial cells. In this in vitro study three women with a history of endometriosis who were referred to the infertility clinic of Royan institute were recruited. All cases signed an informed consent form. This study was approved by the ethical committee of Royan institute that has been started on May 2018.

Materials and Methods: The polylactic acid granules are solved in the mixture of NaOH with steeled water as a solvent with the ratio of one fifth. Solution was loaded into Quantum 2035 3D printing device with Cura software and MK9 extruder. The printer is one headed whose internal diameter of the head is changeable about 0.4 to 1mm.The final product size is 24*20*35 cm with the 100-micron accuracy and the thickness of the layer is about 60-400 micron. The speed of print is about 120 mm in sec, and the speed of head movement and the temperature of head is 180 mm in sec and 270^{o}, in order. The primary endometrial cell culture on this scaffold was performed using DMEM/F12 with 10% FBS culture medium plus 1% Pen-Strep up to 50% of confluence. The participants in this investigation were 3 persons who were under sampling by using piple for the endometriosis reason.

Results: We could produce 3D uterus shape scaffold, which is made of Polylactic acid that is porous, cell friendly, biocompatible and biodegradable. The initial endometrium primary cell culture on this scaffold was successfully performed. Our primary results are compatible with previous studies, which were used different scaffolds for 3D culture of endometrial cell. However, our scaffold was much more similar to in vivo study anatomically. In addition, it seems the function of this scaffold is more applicable than others that were used in endometrial 3D culture.

Conclusion: Therefore, we concluded that our 3D uterus shaped scaffold could be used as a perfect in vitro model for endometrial culturing in future studies. This model will be much more similar to the in vivo investigations than others including 2D culturing systems.

Key words: *3D printing, Tissue engineering, Reproductive system, Uterus, PLA.*

A-6
Evaluation of the structure and ultrastructure of fresh and frozen/thawed ovarian tissue in cancer patients, after chick embryo chorioallantoic membrane

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Background: With the improvement in diagnosis and treatment, the survival rate of cancer patients is increasing. But, ovarian tissue (OT) that contains irreplaceable resources is susceptible to cytotoxic effects of chemotherapy drugs. The ovarian cryopreservation is a promising alternative in fertility preservation programs. Ovarian cortex can be obtained by using laparoscopic surgery at any stage of the menstrual cycle before starting cancer treatment.

Objective: The aim of this study was evaluation of the structure including follicle morphology and stroma and ultrastructure of fresh and frozen/thawed OT after two methods of vitrification and slow freezing, in cancer patients, after 5 and 10 days grafted onto chick embryo chorioallantoic membrane (CAM) And determining the best freezing method for ovarian tissue in patients who are seeking fertility preservation and also ensuring that the metastasis cell is not frozen in the tissue.

Materials and Methods: Small pieces of the OT from 10 cancer patients, under 30 yr, were included in the study after obtaining written informed consent. These pieces were divided into three general groups of fresh (control), vitrification and slow freezing. These components were controlled by the presence of metastatic cells with stained slides, by an experienced pathologist. After grafted onto the CAM, they were removed after 5 and 10 days. These components were divided into two groups based on the addition of activin to the culture medium. Structural and

ultrastructural studies were done for assessment of follicular integrity.
Results: The mean age for participant was 26.7 and none of the OT had metastatic cells. After 5 days of culture, follicogenesis occurred in the presence of activin ($p<0.05$), but in the long-term culture, it was independent ($p<0.05$). The quality of follicles in the non-transplanted tissue was better than the two freezing groups ($p<0.05$). Long-cultured transplantation had a negative impact on follicle and stromal quality. Expression of PCNA in granulosa cells was started at intermediate follicles stage. The vitrification was more successful in transplantation than slow freezing (65% vs. 59%). In the ultrastructure study, better follicle quality was obtained in slow freezing and better stroma in vitrification group. Activin was identified as a follicle activator and also anti-angiogenic factor.
Conclusion: At the end of a 10-days culture, the follicles had better morphology in the tissue from slow freezing and better stromal cells in vitrification. CAM is suitable environment for short-term tissue transplantation. Also, activvin did not improve the culture condition for OT.
Key words: *Fertility preservation, Human ovary, Transplantation.*

A-7
Investigation of stemness state in human amniocytes

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Background: Many studies have described amniotic fluid-derived stem cells (AFSCs) as a fascinating source of stem cells. Differentiation ability to all three embryonic germ layers, immunomodulatory properties, lacking tumorigenicity and less ethical and legal limitations rather than embryonic stem cells introduce an exciting choice for the future of regenerative medicine.
Objective: The present paper aims to validate the expression of some stemness markers in 10 amniotic fluid cell lines. The genes investigated as stemness markers include OCT4, NANOG, SOX2, c-KIT (CD117), c-MYC, KLF4, FGF4 and THY1 (CD90).
Materials and Methods: Amniotic fluid samples were donated by genetics laboratories of Yazd reproductive sciences institute. The amniocytes were cultured with a modified medium composed of 2:1 v/v DMEM: AmnioMAX II in which DMEM was supplemented by 4 mM L-Glu, 10 mM HEPES, 15% FBS, 1% PenStrp. Culture vessels were incubated at 37^{o}C under 5% humidified CO_2. For RT-PCR, amniocytes were harvested by 0.25% Trypsin-EDTA and total RNA was isolated using the Qiagen RNeasy™ Mini Kit. cDNA synthesis was performed by means of the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) and finally, cDNA samples were amplified by human-specific primers.
Results: The most remarkable results to emerge from the data is that all 10 amniotic fluid cell lines at 4th passage expressed at least one of the stemness markers. The OCT4 marker was positive in 6 samples and NANOG in 4 samples. All cell lines were negative for SOX2 as well as FGF4. Other interesting results have been revealed by c-KIT marker that was positive only in 2 lines.
Conclusion: The findings of this study indicate the idea that amniotic fluid cells have an intrinsic nature of heterogeneous phenotype and stemness potential that make them a valuable candidate to be used in the field of regenerative medicine.
Key words: *Amniotic fluid cell, Amniocyte, regenerative medicine, Stemness marker.*

A-8
Clinical outcomes of IMSI and embryo morphokinetics in patients with male factor infertility

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Background: Sperm morphology and fine integrity of sperm nuclei are positively associated with fertilization, implantation and clinical pregnancy rates.
Objective: The objective was to evaluate the effect of IMSI on the morohokinetics of early developmental events of embryos and clinical outcomes in patients with different etiologies of male factor infertility.
Materials and Methods: The study population consisted of 75 couples involved in the IMSI program with different etiologies of male infertility: (astenozospermia (AS) n=7, oligoastenotratozospermia (OAT) n=26, astenotratozospermia (AT) n=20, and tratozospermia (T) n=22) patients. The sperm selections were done according to MSOME criteria by Cassuto method, MII oocytes were injected and the zygotes were cultured in time-lapse monitoring system (TLM) for 3 days. The main outcome measures were embryo kinetics and clinical outcomes.
Results: 320 embryos were developed and assessed using TLM. 90 embryos were assessed from OAT, 30 embryos from AS, 92 embryos from AT and 108 embryos from T patients. Data showed, that the rate of cleavage abnormalities and embryo morphokinetic timing had no significant differences between them ($p>0.05$). Among

the transferred embryos in different patients, the rates of chemical pregnancy and implantation were respectively higher in OAT patients (37.8% and 38.2%) and the clinical pregnancy and live birth rates were insignificantly higher in Tratozospermia patients (32% and 32%) when compared with other patient groups.

Conclusion: Sperm selection with MSOME parameters and IMSI can improve the embryo morphokinetics and clinical outcomes in couples with male factor infertility, especially for OAT and tratozospermia patients.

Key words: *MSOME, IMSI, Male infertility, Time lapse, Pregnancy.*

A-9

Medium containing different concentrations of catalase as a strategy for optimizing sperm parameters and chromatin in normospermic persons

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Background: Aerobic metabolism of human sperm produces reactive oxygen species (ROS). During sperm preparation. antioxidant remove from semen This damage. change in the sperm motility and DNA damage. ontrol of lipid peroxidation is exerted by antioxidant. one of important antioxidant is catalase. Studies show that the addition of catalase to the sperm media effect on sperm function.

Objective: Therefore, the aim of this choose the best concentration of catalase for optimizing sperm parameters and chromatin in normospermic persons.

Materials and Methods: Semen samples were selected from normozoospermic men. The semen of each person was divided into eight groups: one group as control and seven as treatment groups. Spermatozoa were prepared by swim-up method for each group.In the treatment groups, different concentrations of catalase (200, 150, 100, 50, 10, 1 and 0.1 IU/mL) were added. In all groups the semen was incubated for 1 hr. After incubation, percentage of motility, viability and morphology of spermatozoa as well as chromatin quality were assessed for all groups. After this time, these tubes were incubated at room temperature. The sperm motility was only analyzed after 4 and 24 hr.

Results: Sperm motility in one hour after exposure to different concentrations of catalase showed no significant difference between groups. However, those spermatozoa exposed to 100 IU/ml of enzyme had the highest motility (71%±8.431 vs. 58%±10.03, p=0.041). Sperm motility after 4 and 24 of exposure to 100 IU/ml of enzyme at room temperature was similar to one hour. However, chromatin quality had the best condition in low concentration of catalase (p=0.015).

Conclusion: This study showed that concentration of 1 IU/ml of catalase should be used to improve parameters and sperm chromatin because at low concentrations, sperm histone replacement by protamines may improve sperm motility in addition to protecting sperm DNA from damage. Therefore, we recommend adding low concentration of catalase (1 IU/ml) to the sperm medium to optimize their movement and protect chromatin structure and integrity.

Key words: *Catalase, Chromatin, Motility, Sperm, Viability.*

A-10

The effect of air dust pollution on semen quality and sperm parameters among infertile men in west of Iran

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Background: Pollutants during haze and Asian dust storms are transported out of the Asian continent, affecting the regional climate and the hydrological and biogeochemical cycles. Nonetheless, no specific studies evaluated the dust particles influence on semen quality in a specific geographical area.

Objective: In this article, we investigated the effect of dust particles on semen quality and sperm parameters among infertile men.

Materials and Methods: A descriptive-analytic study was conducted among 850 infertile men between 2011 and 2015 years. Semen quality was assessed according to the WHO 2010 guidelines, including sperm concentration, progressive motility and morphology. 4-year average dust particle concentrations were estimated at each participant's address using the Air Pollution Monitoring Station affiliated with the Department of Environment of Kermanshah city were gathered.

Results: Dust particle levels were highest in the summer months, in Kermanshah province. Semen analysis data showed that values of dust particles were negatively correlated to sperm morphology and sperm concentration before and after lab-processing, but sperm progressive motility is low sensitive to dust particles.

Conclusion: Our findings showed that exposures to dust particle may influence sperm quantity in infertile men, consistent with the knowledge that sperm morphology and concentration are the most sensitive parameters of dust pollution.

Key words: *Male infertility, Dust particles levels, Air dust pollution, Asian dust storms, Sperm quality.*

A-11

Comparison of oocyte maturation trigger using follicle stimulating hormone plus human

chorionic gonadotropin versus hCG alone in ART cycles

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Background: The success rates of assisted reproduction technology (ART) could be developed with the improvement of ovarian stimulation protocols as well as the optimization of final oocyte maturation.
Objective: The goal of this study was to compare oocyte maturation, fertilization and pregnancy rates among women with concomitant FSH administration at the time of hCG trigger and the hCG trigger alone.
Materials and Methods: In a randomized controlled trial 109 infertile women at the age between 20 to 40 yr, underwent GnRH antagonist protocol and fresh embryo transfer. They were randomly divided into two groups on the day of trigger. In the first group, final oocyte maturation was done by 5000 IU hCG (Pregnyl, Organon, Netherlands) plus 450 IU FSH (Cinnal-f Cinnagen, Iran). In the control group, oocyte triggering was performed by 5000 IU hCG alone. The primary outcome was clinical pregnancy and the secondary outcomes included oocyte recovery rate, oocyte maturity rate, fertilization proportion , fertilization rate, implantation rate and chemical pregnancy rate.
Results: 54 women were allocated to the group with the FSH bolus injection at the time of hCG administration and 55 women assigned to the hCG alone administration group. Women in the FSH group had a significantly higher MII oocyte (7.17±3.50 vs. 5.87±3.19), 2PNs (5.44±3.20 vs. 3.74±2.30) and total embryos (4.57±2.82 vs. 3.29±2.13) compared to hCG alone group respectively. Furthermore, fertilization rate (0.75±0.19 vs. 0.68±0.25), implantation rate (14.2% vs. 8.5%) as well as clinical (27.9% vs. 15.9%) and chemical (32.6% vs. 20.5%) pregnancy rates were higher in FSH group, but no statistically significant difference was found ($p>0.05$).
Conclusion: Combination of FSH and hCG for oocyte triggering improves oocyte maturity and fertilization proportion rate without increasing the chance of implantation, chemical and clinical pregnancy rates.
Key words: *Oocyte triggering, FSH, hCG, ART outcome.*

A-12
Outcome of assisted reproductive technology in different subgroups of poor ovarian responders fulfilling the POSEIDON criteria

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Background: The population of poor ovarian responders among patients undergoing in vitro fertilization (IVF) has been suggested to vary between 9 and 25%.
Objective: Low success rates are common in women undergoing in vitro fertilization who respond poorly to ovarian hyperstimulation. Due to the heterogeneity of the populations, expressing a unique and shared definition is necessary in order to individualize infertility treatment. The main goal of this study was to evaluate the assisted reproductive technology outcome among various subgroups of poor responders defined by the POSEIDON (Patients-Oriented Strategies Encompassing IndividualizeD Oocyte Number) stratification.
Materials and Methods: In this retrospective cohort study, the clinical and laboratory records of 245 poor responder women undergoing their first ovarian stimulation and fresh embryo transfer cycle were reviewed. Patients were categorized into 4 groups according to the POSEIDON classification.
Results: The fertilization rate was comparable between groups ($p>0.05$). Moreover, there was no difference in implantation rates between groups 1 and 2 and groups 3 and 4 ($p>0.05$). In contrast, chemical and clinical pregnancy rates, as well as live birth, were significantly higher in group 1 and 2 compared to the groups 3 and 4 ($p<0.05$). As regards POSEIDON subgroup stratification, there were not significantly different between four subgroups of group 1 and 2 on the subject of ART outcomes ($p>0.05$).
Conclusion: Future studies should explore the most optimal treatment strategy for poor responders according to the POSEIDON stratification and suggested handling with live birth as primary end-point.
Key words: *ART outcome, Poor responder, POSEIDON stratification, Individualized treatment.*

A-13
One-carbon cycle support rescues sperm damages in experimentally induced varicocele in rats

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Background: Varicocele is one of the most prevalent causes of infertility. It causes induction of oxidative stress, increases lipid peroxidation and DNA fragmentation in the testis and disrupts spermatogenesis cycle. Balanced diet is the natural source of micronutrients, such as folate and vitamins, vital for proper functioning of the body.
Objective: To demonstrate that micronutrients in support of the one-carbon cycle and glutathione synthesis are effective in improving sperm damage following surgical varicocele in rats and that this effect is achieved without suffering a rebound reductive stress as seen with oral antioxidants.

Materials and Methods: Surgical varicocele was induced in adult male Wistar rats and resulted in significant damage to testis and sperm cells measured 2 and 4 months post-surgery. Two months after surgery, rats received a 2-month oral supplementation in support of the one-carbon cycle containing B vitamins (B2, B3, B6, folic acid and B12), N-acetyl-cysteine, zinc, small amounts of Vitamin E and a natural source of betalains and quercetine (Condensyl).
Results: One-carbon cycle supplementation, compared to untreated controls, significantly improved the morphometric characteristics of testis ($p<0.05$), sperm concentration, motility and abnormal morphology ($p<0.001$), sperm chromatin condensation (aniline blue staining, $p<0.05$), sperm DNA damage (acridine orange staining, $p<0.05$) and sperm lipid peroxidation (BODIPY C11, $p<0.001$). The improvement of both nuclear condensation and DNA damage and the lack of excessive inhibition of lipid peroxidation confirmed that no reductive stress had occurred.
Conclusion: Micronutrients in support of the one-carbon cycle are effective in the treatment of the rat experimental varicocele, likely by activating the natural antioxidant defenses and epigenetics. These results support the idea that essential micronutrients Accepted Article This article is protected by copyright. All rights reserved. Including B vitamins may have a positive influence also in clinical varicocele, which should be tested in prospective clinical trials.
Key words: *Condensyl, DNA damage, Lipid peroxidation, Nuclear condensation, Varicocele.*

A-14

Intra-testicular injection of autologous extracellular vesicle from adipose tissue mesenchymal stem cell (EV-ADMSC) in non-obstructive azoospermia Mice

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Background: Azoospermia affects about 1% of the male population and may be seen in around 20% of male infertility conditions. In testicular azoospermia the testes are abnormal, atrophic, or absent, and sperm production severely disturbed to absent. FSH levels tend to be elevated (hypergonadotropic) as the feedback loop is interrupted. The condition is seen in 49-93% of men with azoospermia. Non-obstructive azoospermia (NOA) is generally considered a non-medically manageable cause of male infertility. A number of in vitro studies confirmed that mesenchymal stem cells (MSCs) could differentiate into germ cells. MSCs injection reconstructed testicular germinal epithelium in infertile male animal models. In addition, some studies reported that MSCs could differentiate into sperm and regenerate spermatogenesis. There's is a limited number of published clinical trials in this regard which showed the promising results of this approach in treating male infertility.
Objective: We hypothesized that Extracellular Vesicle from Adipose Tissue Mesenchymal Stem Cell (EV-ADMSC) are useful in treating NOA.
Materials and Methods: Adult male mice were randomly divided into three groups including the intact control group, azoospermia-induced group, and Conditioned Media (CM) of Adipose Tissue Mesenchymal Stem Cells (AT-MSCs) therapy. Except intact control the other groups were intraperitoneally received two doses of 10 mg/kg of busulfan with 21 days' interval in order to induce azoospermia. AT-MSCs were isolated from subcutaneous fat tissue of donor mice. The CM of AT-MSCs were injected into the efferent tubules of testes of treatment group 35 days after the last busulfan injection. The cell therapy groups were sampled 60 days after cell therapy and the azoospermia group and azoospermia and control groups were sampled in the same date. The histomorphometric indices of testes were evaluated.
Results: Histopathologic evaluation of testes showed that most of the seminiferous tubules of CM therapy group had normal morphology and showed spermatogenesis. Spermatogenesis was not observed in the azoospermia group.
Conclusion: CM of AT-MSCs of mice recovered spermatogenesis in the seminiferous tubules of busulfan-induced azoospermic testes. Therefore, CM of AT-MSCs can be suggested as a candidate in CM therapy of azoospermia.
Key words: *Non-obstructive azoospermia (NOA), Extracellular vesicle, Mesenchymal Stem Cells, Spermatogenesis.*

A-15

The effect of myo-inositol on the oocyte quality, fertilization rates and signaling pathway of steroidogenesis in PCOs women undergoing ART cycles

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Background: Polycystic ovarian syndrome (PCOS) is the most common cause of infertility, steroidogenesis and metabolic impairments, ovarian dysfunction and menstrual irregularity, affecting 5-10% of women in reproductive age. Inofolic is a combination of myo-inositol and folic acid, which improves pathological conditions associated with PCOS and improves fertility. Recently many authors have investigated whether supplementation with inofolic, improved the oocytes' quality and increase the number of oocytes collected after ovarian stimulation in patients with PCOS undergoing in vitro fertilization. But so far, no study has examined the signaling pathway of inofolic on steroidogenesis in these patients.
Objective: The aim of this study was to evaluate the effects of inofolic on oocyte quality, fertilization rate, embryo quality and steroidogenesis signaling pathway in cumulus cells of PCOS women undergoing ART cycles.
Materials and Methods: Forty infertile PCOS patients were randomly designated into two groups and twenty cases was normal (control). In the first group, PCOS patients received daily doses of 4g Myo-Inositol combined with 400mg folic acid and in other group patients received only 400mg folic acid from one month before starting the antagonist cycle until the day of ovum pick up. Normal group also received only 400mg folic acid from one month before starting the antagonist cycle until the day of ovum pick up. Oocytes and embryos quality were assessed according to European Society of Human Reproduction and Embryology (ESHRE) guidelines. The gene expression FSHR, CYP11A1, CYP19A1, ER, StAR, 3β-HSD2 in cumulus cells were analyzed using real-time RT-PCR.
Results: The percentage of metaphase II oocyte, fertilization rate and embryo quality significantly improved in the study group which received inofolic ($p<0.05$), but the number of retrieved oocytes and follicle count were not statistically different between groups. Furthermore, the gene expression of FSHR, CYP11A1, CYP19A1, ER, StAR, 3β-HSD2 were significantly higher in the study group ($p<0.05$).
Conclusion: The findings of our study provides some new molecular evidence about the possible mechanism of Myo-Inositol effect on the fertilization rate, quality of oocytes and embryos. This might be related to modification of steroidogenesis pathway in cumulus cells of PCOS women undergoing ART cycles.
Key words: *Polycystic ovary syndrome, Infertility, Myo-Inositol, Assisted reproductive technology.*

A-16

Profile of apoptotic and cell cycle genes in the follicular fluid of patients with the empty follicle syndrome who is treated by GnRH antagonist protocol

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Background: Alternation of follicular fluid component induced by paracrine, and autocrine signaling pathways may influence the oocyte quality. Various genetic factors have been previously demonstrated for the development of empty follicle syndrome (EFS).
Objective: Present study aimed to investigate gene expression profiles of apoptosis and cell cycle pathways using microarray technique in the follicular fluid (FF) of 10 patients with genuine EFS (GEFS) undergoing GnRH antagonist protocol.
Materials and Methods: In this randomized clinical trial study, FF of the empty follicles were collected from 10 infertile women following first and second triggering with hCG. RNA was extracted from the FF samples containing oocytes and without oocytes for gene expression analyses. PCR arrays were performed to evaluate gene expression profiles related to the apoptosis and cell cycle pathways. PCR array validation was carried out by qPCR.
Results: Remarkable alternation were observed in the apoptotic gene expression including BCL2, COMP, GADD45A, IL1B, MSX1, TNF and TNFRSF10B in the FF from patients who had oocytes retrieved when compared to the group without oocyte. Moreover, differentially expression of genes related to the cell cycle pathway comprising CCNB1, GADD45A, MKI67, STMN1 and TGFBβ1 were detected in the patients who had oocytes retrieved in comparison to those without oocyte. Changes in the gene expression of both two pathways quantified by q RT-PCR were in agreement with the microarray findings. This study demonstrated that aberrant apoptosis and cell cycle signaling molecules expression might be considered as a genetic basis for EFS.
Conclusion: Therefore, it can be concluded that properly function of these genes within the apoptosis and cell cycle pathways might culminate to the final maturation of oocytes which is required for a successful fertilization in IVF treatment.
Key words: *Empty follicle syndrome, Apoptosis, Cell cycle, Antagonist protocol, PCR array, Follicular fluid, Oocyte.*