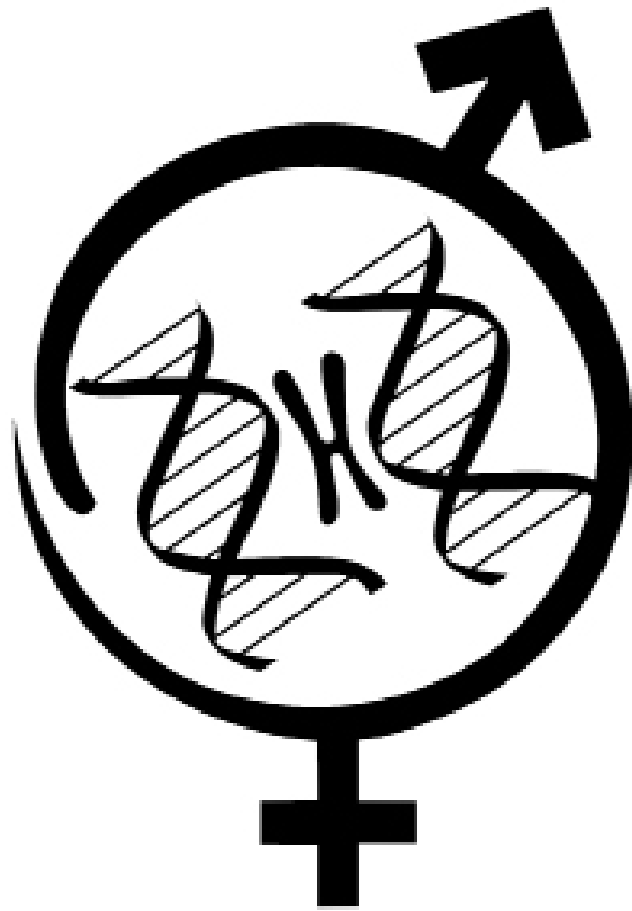


Abstracts of

Royan International Twin Congress

14th Congress on Stem Cell Biology and Technology
29-31 August 2018



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran



**Abstracts of the 14th Congress on
Stem Cell Biology and Technology (2018)**

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Sara Pahlevan

Dear Colleagues,

It is our pleasure to invite you to participate **in The 14th International Congress on Stem Cell Biology and Technology** taking place on **29-31 August 2018** in **Tehran, Iran**.

Following the success of the previous events, this 14th Congress will also address areas of stem cell science from bench to market in an exciting and informative manner. With world renowned speakers secured to present ground breaking news, Royan 14th International Stem Cell Biology and Technology will be a congress to be remembered and definitely not to be missed.

We know how important advances in stem cell science are. As the leading professional society for stem cell research in Iran, we are committed to building a network with international leaders in the stem cell field as a way to promote and foster the exchange of information and research.

Participants will enjoy cutting-edge lectures and very active discussions by the world's top scientists, senior researchers and postgraduate students. Topics to be covered, among others will be:

1. Current and future trends on stem cell science in
 - Pluripotent Stem Cells and Adult Stem Cells
 - Stem Cells in Cardiovascular Research
 - Stem Cells in Neuroscience
 - Stem Cells in Internal Organs Research
 - Stem Cells in Skin and Derivatives Research
 - Stem Cells in Bone and Cartilage Research
 - Stem Cells in Reproduction
 - Stem Cells in Cancer Research
 - Bioinformatics in Stem Cell Research

2. Stem Cells and Regenerative Medicine

Join the Congress and benefit from expanding your network. Meet new friends, greet old colleagues and discuss new insight gained.

We wish you a fruitful and enjoyable congress and looking forward to welcoming you to Tehran.

Sara Pahlevan, Ph.D.
Congress Chairperson
14th Congress on Stem Cell Biology & Technology

Invited Speakers

Is-1: Myeloid Lineage Cell during Healing: Friend or Foe?

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Background: Inflammation, following skin injury, initiates a series of autocrine and paracrine signals as well as a fine-tuned cellular interplay. Myeloid cells are one of the leading players in this orchestrated phenomenon.

Materials and Methods: Using *in vitro* and *in vivo* models of injuries, we highlight the contribution of myeloid cells as well as their fate during skin healing. We evaluate this role in different models of healings, including deficient and excessive healing.

Results: While myeloid cells show an essential role in normal skin healing, disturbed myeloid cells contribute to the deficient healing of aged animals. Conversely, the augmented and sustained function of myeloid cells contributes to excessive healing, leading to scar formation.

Conclusions: These works introduce a paradigm in which myeloid cells are recognized as the significant player in granulation tissue formation and skin healing.

Is-2: 3D Skin Printing: In-Situ Formation of Planar Tissues

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Background: Wound healing and coverage are the crucial determinants of burn patient survival. However, these are limited by factors such as limited skin availability, size, and cell availability. Conventional wound dressings merely protect from infection and absorb exudate fluid as passive layers. Of the current treatments for skin, autografts are limited in the extent of available tissue, cell cultured epithelial autografts (CEA) grafts have a limited thickness and mechanical stability, and synthetic skin grafts (such as collagen/silicone bilayers) have slow cell infiltration. There is a significant potential for wound dressings as active devices to dynamically participate in the wound repair process through cell and scaffold delivery to the wound.

Materials and Methods: A skin printer that enables the in-situ formation of biomaterial and skin tissue sheets of different homogeneous and architected compositions will be introduced.

Results: Consistent sheet formation is achieved by coordinating the flow rates at which bioink and cross-linker solution are delivered, with the speed at which a pair of rollers actively translate the cartridge along the surface. Upon rapid crosslinking, biomaterial and skin cell-laden sheets of consistent thickness, width and composition were obtained. Proof-of-principle demonstrations for the in-situ formation of biomaterial sheets in murine and porcine excisional wound models illustrate the capacity of depositing onto inclined and compliant wound surfaces that are subject to respiratory motion.

Conclusions: The presented work will enable the in-situ delivery of a wide range of different cells, biomaterials, and tissue

adhesives, as well as the in-situ fabrication of spatially organized biomaterials, tissues, and biohybrid structures.

Is-3: Corneal Regeneration

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Limbal stem cells (LSCs) are regenerative engine for continuous corneal epithelialization. Destruction of these stem cells or their dysfunctional niche will lead to limbal stem cell deficiency (LSCD). Limbal stem cell transplantation (LSCT) is the definitive treatment for LSCD. In unilateral cases, Conjunctival-Limbal autograft transplantation (CLAU) and Cultivated Limbal Epithelial Transplantation (CLET) are the classical modalities. In bilateral cases, Keratolimbal Allograft Transplantation (KLAL), Living-Related Conjunctival-Limbal Allograft Transplantation (Lr- CLAL) and Cultivated Oral Mucosal Epithelial Transplantation (COMET) are surgical alternatives. In a recent publication, we showed that *in vivo* cultivation of LSCs using amniotic membrane as a niche and amniotic membrane extract eye drop (AMEED) as a promoting factor is a good surgical alternative in the case of unilateral LSCD. Now, in bilateral cases we are working on *in vivo* cultivation of oral mucosal epithelial cells using amniotic membrane transplantation and AMEED. Also, we are working on *in vivo* cultivation of LSCs using E-PRP drops. Our preliminary results are promising.

Is-4: Two-Way Conversion between Lipogenic and Myogenic Fibroblastic Phenotypes Marks The Progression and Resolution of Lung Fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is a form of progressive interstitial lung disease with unknown etiology. Due to a lack of effective treatment, IPF is associated with a high mortality rate. The hallmark feature of this disease is the accumulation of activated myofibroblasts that excessively deposit extracellular matrix proteins, thus compromising lung architecture and function and hindering gas exchange. Here we investigated the origin of activated myofibroblasts and the molecular mechanisms governing fibrosis formation and resolution.

Materials and Methods: Genetic engineering in mice enables the time-controlled labeling and monitoring of lipogenic or myogenic populations of lung fibroblasts during fibrosis formation and resolution.

Results: Our data demonstrate a lipogenic to- myogenic switch in fibroblastic phenotype during fibrosis formation. Conversely, we observed a myogenic-to-lipogenic switch during fibrosis resolution. Analysis of human lung tissues and primary human lung fibroblasts indicates that this fate switching is involved in IPF pathogenesis.

Conclusion: These results open the way for potential therapeutic avenues to treat IPF patients.

Keywords: IPF, Lipofroblast, Myofibroblast, Lineage Tracing, Fgf10

Is-5: Imprinting Cell Membrane: Recent Progress and Future Perspective

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Imitating the natural environment of isolated cells is well believed as an important criteria for regulation of cell fate. Previous reports showed that cell imprinted substrates can offer invaluable simple means of investigation in cell differentiation. In this method, micro/nano-scale features of cell membrane are transferred to a culture surface. Afterwards, this substrate is utilized as substitute for prevalent polystyrene tissue culture plate. It was observed that cell imprinted substrates can be utilized for induction of chondrogenic, keratinogenic, tenogenic, myogenic and osteogenic differentiation in adipose derived mesenchymal stem cells. In addition, the results also showed promising outcomes in migration, proliferation and differentiation of other cell types. Therefore, safety and efficiency of cell imprinting method can enhance the quality of tissue regeneration both in cell free and cell based strategies. Taking together, cell imprinting method can be applied to improve the development of next-generation stem cell culture materials and implant interfaces.

Is-6: Consequences of Ribosome Biogenesis Inhibition on Normal and Pathological Intestinal Stem/Progenitor Cells

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Today, ribosomes are no longer viewed as having a passive role in protein translation but rather being a multifaceted machinery involved in the spatiotemporal control of gene expression in various contexts including development, stress or disease. Human genetics has largely participated to this shift of paradigm through the discovery that mutations in gene coding for core components of the ribosome cause tissue-specific developmental phenotypes. In vertebrates, ribosome biogenesis (RiBi) and p53 levels are intricately connected through a surveillance pathway, as imbalance between rRNA and ribosomal protein synthesis leads to p53 activation. Deregulated RiBi is strongly associated with diseases and notably cancer. Non-genotoxic inhibitors of RiBi represent novel and promising therapeutic tools to target the translation apparatus of cancer cells. While this approach holds great promise as therapeutic strategy, a comprehensive understanding of the consequence of ribosome biogenesis inhibition on cancer cells requires further *in vivo* investigations. In my team, we are interested in understanding how RiBi is regulated *in vivo* and how perturbations of this process impacts on normal and pathological tissue homeostasis. We are addressing this question using the mouse as a model system through the analysis of the Notchless (Nle) gene, coding for a critical RiBi factor required for pre-ribosomal 60S subunit

maturation and export. I will present our recent findings that different responses to RiBi inhibition are elicited depending on cell type and context. I will also discuss about the relevance of targeting ribosome biogenesis in Wnt-driven tumorigenesis.

Is-7: Specification and Maintenance of Cell Identities in the Early Mammalian Embryo

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A unique paradigm for the analysis of cell fate decisions *in vivo* with high spatiotemporal resolution in mammals is the preimplantation mouse embryo. We are focusing our research on the formation of pluripotent epiblast (Epi) and extra-embryonic primitive endoderm (PrE) cell lineages within the inner cells of the blastocyst. Initially, uncommitted inner cells co-express markers of both lineages. Epi and PrE are then asynchronously specified within the inner cell mass of the blastocyst in a salt and pepper pattern and become spatially segregated before the implantation. We are investigating the contributions of candidate factors and regulatory mechanisms using both pharmacological and genetic perturbations on mouse embryos combined with 3D live imaging and lineage specific fluorescent reporters. I will present our recent data on the temporal dynamics of Epi and PrE progenitor emergence and the mechanisms controlling progenitors maintenance after their specification.

Is-8: Using Epidermal Neural Crest Stem Cells in Experimental Models of Spinal Cord Injury

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Spinal cord injury (SCI) and its attendant neurodegenerative processes is a serious health issue in which the current surgical and pharmacological management approaches fail to fully overcome the devastating condition. A search through experimental and undergoing clinical trials reveal that cell-based therapies using a variety of embryonic stem cells, adult mesenchymal stem cells and induced pluripotent stem cells are active fields of research in SCI treatment. The mechanisms through which the cells may repair the injured spinal cord are immunomodulation, providing a trophic support and/or replacing the injured neurons and glia to restore the architecture and function. However, these attempts have not yet been sufficiently successful to achieve the regulatory approval as standard and popular treatment goals. Although this failure may partly reflect the neglecting complex neuropathology of SCI which necessitates multi-target therapeutic approaches, can be also attributed to the source of stem cells making not adequate therapeutic concentration or accompanying adverse reactions. Therefore, searching for more appropriate sources of stem cells and combining the cell-based therapies with other approaches to manipulate the inhospitable context of injured tissue and strengthen the cells are of great importance. Epidermal neural crest stem cells (EPI-NCSCs) are multipotent adult stem cells that originate from the dorsal developing neural

tube, reside in the bulge of hair follicles and persist postnatally into adult life. These cells were first introduced by Sieber-Blum et al., in 2004, isolated from bulge explants of adult mouse whisker follicles. Thereafter, EPI-NCSCs were also isolated and characterized from rat, canine and human. In addition to high degree of innate plasticity of EPI-NCSCs, the migratory behavior of these cells which yields a high pure population of stem cells, and their accessibility through minimal invasive procedures have all established them as potential candidate in autologous cell-based therapies.

Using *ex vivo* (organotypic slice culture) and *in vivo* models of traumatic SCI, we currently evaluate the efficacy of rat EPI-NCSCs combined with pharmacological agents. The drugs are selected based on potential effects on the survival, proliferation and differentiation of isolated stem cells and in parallel modulating the host tissue. Based on our results, valproic acid, a widely used antiepileptic drug, which acts as a histone deacetylase inhibitor and fingolimod, an immunomodulating drug for treating multiple sclerosis, which acts as a sphingosine 1-phosphate receptor modulator are introduced as promising candidates for EPI-NCSC combinatorial treatments of SCI.

Keywords: Epidermal Neural Crest Stem Cells, Spinal Cord Injury, Valproic Acid, Fingolimod

Is-9: Impact of Sperm Hyaluronidase and VLMWHA on Sheep Blastocyst Formation *In Vitro*, Viability after Cryopreservation and Pregnancy Rate after Embryo Transfer

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Background: Recent research in our laboratory has reported the presence of members of the hyaluronan (HA) system including HA synthases and receptors and hyaluronidase (hyals) in reproductive system and embryos and the critical role of *hyal2* in early stage embryo development. We hypothesised that very low molecular size HA fragments (VLMWHA; <10kDa) produced during degradation by sperm hyaluronidase (PH20), function as a survival factor and growth stimulator during pre-implantation embryo development.

Materials and Methods: Sheep oocytes were collected from slaughterhouse derived ovaries and matured and fertilized *in vitro*. Experiment 1: Cleaved embryos were cultured in the absence (control) or presence of 10ng/ml PH20, or 100µg/ml VLMWHA or anti HA cell membrane receptors CD44 and RHAMM for 6 days when development to blastocyst was recorded and the number of hatched blastocysts counted. Experiment 2 assessed quality of the blastocysts based on survival after cryopreservation by vitrification of early blastocyst stage embryos. Experiment 3 analysed pregnancy and live birth rates after embryo transfer to oestrus synchronised recipient ewes. Pregnancy was assessed by ultrasound scanning on day 35, and number and normality of lambs were recorded.

Results: Significantly higher percentage of blastocysts were produced in PH20 (56.8 ± 6.9) or VLMWHA (63.6 ± 4.0) versus control (32.4 ± 3.4 %). Similarly higher proportion of these blastocysts were hatched (PH20; 21.6 ± 3.1 , VLMWHA; 22.6 ± 4.4 , Control; 7.2 ± 1.2) $P < 0.05$). These effects were abrogated in the presence anti-CD44 and RHAMM. Higher percentage of the blastocysts cultured in PH20 or VLMWHA survived after vitrification as observed by re-expansion and hatching after re-

culture (76.2% and 80% v. 52.2%, $P < 0.05$). Higher number of pregnancies and live birth was observed in the ewes receiving blastocysts developed in the presence of PH20 (8/11; 73%) or VLMWHA (9/12; 75%) versus control (6/11; 55%). No abnormality was observed in the lambs weight, behaviour and survival.

Conclusion: These studies have defined a new a new role for sperm in supporting early stage embryos and provided evidence for a receptor-mediated role of sperm hyaluronidases or VLMWHA in enhancing embryo development and quality during the preimplantation period.

Keywords: Hyaluronan, Hyaluronidase, Sperm, Blastocyst, Pregnancy and Live Birth

Is-10: Towards The Use of Endometrial Mesenchymal Stem Cells as A Cell-based Therapy

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We are using a tissue engineering approach to address problems associated with using vaginal mesh for treating POP. We propose to use autologous endometrial mesenchymal stem cells (eMSC) delivered on polyamide/gelatin composite meshes (eMSC/PA+G) to improve mesh biocompatibility and regenerate vaginal tissue damaged from childbirth injury.

eMSC are purified from biopsies obtained from premenopausal and short-term estrogen-treated post-menopausal women using SUSD2 magnetic-bead sorting. eMSC are culture-expanded in serum-free medium under hypoxia with a small molecule TGFβ-receptor inhibitor, A83-01, generating 90-95% SUSD2⁺ eMSC. ATACseq and RNAseq revealed that A83-01 maintains eMSC stemness by opening chromatin loci enriched for transcription factor binding sites and upregulating gene networks involved in developmental and stem cell signalling pathways.

A rat model treated with human eMSC/PA+G constructs showed that eMSC increased vascularisation, reduced chronic inflammation, promoted deposition of crimped collagen, generating a biomechanically less stiff mesh/tissue complex compared to PA+G. The eMSC had a paracrine mechanism of action in improving biocompatibility of PA+G mesh.

We are using an autologous preclinical ovine model of vaginal surgical repair in multiparous ewes. POP is assessed using a modified POP-Q and our novel fibre-optic pressure sensor device. Ovine eMSC are isolated from hysterectomies by FACS sorting CD271⁺CD49f⁻ cells, culture-expanded and labelled with IODEX-FITC paramagnetic nanoparticles for cell tracking before seeding on PA+G mesh and implanting transvaginally using urogynaecological surgical procedures.

Our tissue engineering approach using autologous eMSC delivered on polyamide/gelatin scaffolds addresses some problems associated with the use of vaginal mesh and may improve surgical outcomes for treating POP.

Is-11: Identification and Characterisation of Endometrial Stem/Progenitor Cells

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Background: To identify specific markers of functional human and mouse endometrial epithelial progenitor cells (eEPs) and mesenchymal stem cells (eMSCs) and determine their identity and location. Our hypothesis was that endometrial stem/progenitor cells would be located in the basalis layer of human endometrium not shed during menstruation, which is responsible for providing cells to repair and regenerate endometrium each month.

Materials and Methods: Hysterectomy tissue was obtained from women who had not been taking hormones for 3 months. Single cell suspensions were prepared by enzyme digestion and mechanical means. A candidate approach to identify human eMSCs using MSC and perivascular markers was used. For human endometrial eEPs, gene profiling of EpCAM magnetic bead sorted pre- and post-menopausal endometrial cells was used to identify differentially expressed adhesion molecules. Clonogenicity, serial cloning and differentiation assays were used to assess the efficacy of candidate markers. In mice, label retention of BrdU and a telomerase reporter mouse (mTert-GFP) were used to identify mouse endometrial eEPs and eMSCs.

Results: Co-expression of CD140b and CD146 enriched for a small population (1.5%) of clonogenic stromal cells with MSC properties and showed their perivascular niche in the basalis and functionalis. A single perivascular marker, SUSD2 comprised 4% of endometrial stromal cells with MSC properties and reconstituted stromal tissue *in vivo*. Of 22 differentially expressed adhesion molecules, 11 were upregulated in basalis-like postmenopausal endometrial epithelial cells, including CDH2 and CDH3. Functional assays showed that clonogenic, self-renewing and organoid-forming epithelial cells were N-cadherin⁺, but not P-cadherin⁺ cells. N-cadherin⁺ cells were located in the gland bases in the basalis. In mice, epithelial label retaining cells (LRC) were located in the luminal epithelium and initiated endometrial regeneration in response to estrogen in estrogen depleted mice. Stromal LRC were near the endometrial-myometrial junction around some vessels. MTert-GFP⁺ cells were predominantly leukocytes, but a small population of luminal epithelial cells and endothelial cells were identified.

Conclusions: Human and mouse endometrium contains small populations of functional epithelial progenitor cells and MSC. Our markers for these stem/progenitor cells allows their quantification in tissue and menstrual blood to assess their role in endometrial proliferative disorders and their purification for further characterisation or use in cell-based therapies.

Is-12: Common Biochemical Origin for Embryonic Stem Cell Death and Differentiation

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Various evidences indicate that differentiation and apoptosis share common features. We aimed to investigate whether

mitochondrial apoptosis and specification employ a common pathway. Our investigation has shown that mitochondria have non-energetic role during differentiation and mitochondrial apoptosis executioners promote differentiation process without being involved in cell death. Using split luciferase complementary assay, we showed that delay in apoptosome complex formation contributes to mouse embryonic stem cells cardiogenic differentiation. Then, we assessed the contribution of apoptosis hallmarks to human embryonic stem cells (hESCs) cardiogenic differentiation. Our results indicate the involvement of mitochondrial attenuated apoptosis-like pathway as well as reversible mitochondrial outer membrane permeabilization in cardiogenic differentiation progression. Moreover, we found that upon doxorubicin induction, the well-known apoptosis inducer, hESCs can be specified for differentiation. Additionally, our further investigation showed that low level of Apaf-1 expression during early stages of neural differentiation can be considered as a possible regulatory barrier by which differentiating cells control cell death upon rise in ROS (reactive oxygen species) elevation and cytochrome c release from mitochondria.

Is-13: Dynamic Control of Embryonic Neural Stem Cells

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During brain development, neural stem cells proliferate intensively while they change their competency over time, giving rise to various types of neurons and glial cells sequentially. It is therefore very important to maintain neural stem cells until the final stage of development to generate a sufficient number of cells and a full diversity of cell types. To understand the mechanism of maintenance of embryonic neural stem cells, we investigated the expression dynamics and functions of Hes and proneural genes. We found that the Notch effector genes Hes1 and Hes5 are expressed by neural stem cells, and that in the absence of Hes1 and Hes5, proneural genes such as Ascl1 and Math3 are up-regulated, accelerating neuronal differentiation and exhausting neural stem cells prematurely. By contrast, in the absence of Ascl1 and Math3, many neurons were missing and, instead, those cells that normally differentiate into neurons adopted the astroglial fate. Thus, proneural genes like Ascl1 and Math3 direct neuronal versus glial fate determination. These results indicate that antagonistic regulation between Notch effectors and proneural genes regulate neural stem cell proliferation, neurogenesis and gliogenesis in the developing brain. In the absence of Hes or proneural genes, the proliferation of neural stem cells was impaired in the developing nervous system, suggesting that both Hes and proneural genes also play an important role in proliferation of neural stem cells.

We next found that both Hes1 and Hes5 are expressed in an oscillatory manner by embryonic neural stem cells, and that Hes1/Hes5 oscillations drive cyclic expression of proneural genes. During neuronal differentiation, Hes1/Hes5 expression disappears and proneural gene expression becomes sustained, while Hes1/Hes5 expression becomes dominant during astroglial differentiation. Furthermore, optogenetic induction of Ascl1 oscillation activates proliferation of neural stem cells, whereas sustained expression of Ascl1 induces neuronal differentiation. Thus, oscillatory versus sustained expression of these factors may be important for their activities. These results also sug-

gest that the multipotent undifferentiated state is controlled by multiple oscillating fate-determination factors, while the fate choice is controlled by sustained expression of a selected fate-determination factor.

Is-13: Dynamic Control of Adult Neural Stem Cells

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There are some neural stem cells in two restricted regions of the adult brain: the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus. Unlike embryonic neural stem cells, these adult neural stem cells are mostly dormant/quiescent, and they only occasionally proliferate and give rise to new neurons. The molecular basis for the difference between embryonic and adult neural stem cells is not well understood. We found that embryonic neural stem cells express *Hes1/Hes5* and proneural genes in an oscillatory manner. When their oscillatory expression is dampened, proliferation of neural stem cells is impaired, resulting in microcephaly, indicating that oscillatory expression of *Hes1/Hes5* and proneural genes is very important for efficient proliferation of neural stem cells. To investigate the expression dynamics and functions of *Hes* and proneural genes in adult neural stem cells, we performed live-imaging and loss-of-function and gain-of-function analyses. We found that in the adult brain, *Hes1* expression is high and sustained, while *Ascl1* expression is negative. Furthermore, induction of sustained *Hes1* expression can inhibit proliferation of embryonic neural stem cells and is sufficient to block neurogenesis and maintain quiescent neural stem cells in the adult brain. By contrast, inactivation of *Hes* genes induces *Ascl1* expression and activates neurogenesis in the adult brain. These results indicate that sustained *Hes1* expression and resultant repression of *Ascl1* may lead to the quiescent state of adult neural stem cells. Thus, *Ascl1* oscillations may be important for activation of adult neural stem cells, suggesting that optogenetic induction of *Ascl1* oscillations may be one of the strategies to activate endogenous neurogenesis in the adult brain.

Is-14: Trimethylation and Acetylation of β -Catenin at Lysine 49 Represent A Key Element in ES Cell Pluripotency

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Wnt/ β -catenin signaling is required for embryonic stem (ES) cell pluripotency by inducing mesodermal differentiation and inhibiting neuronal differentiation, but how β -catenin counter-regulates these differentiation pathways is unknown. Here, we show that lysine 49 (K49) of β -catenin is trimethylated (β -catMe3) by Ezh2, or acetylated (β -catAc) by Cbp. Significantly, β -catMe3 acts as a transcriptional co-repressor of the neuronal differentiation genes *sox1* and *sox3*, whereas β -catAc acts as a transcriptional co-activator of the key mesodermal differentiation gene *t-brachyury* (*t-bra*). Furthermore, β -catMe3 and β -catAc are alternatively enriched on repressed or activated genes, respectively, during ES and adult stem cell differentia-

tion into neuronal or mesodermal progenitor cell lineages. Importantly, expression of a β -catenin K49A mutant results in major defects in ES cell differentiation. We conclude that β -catenin K49 trimethylation and acetylation are key elements in regulating ES pluripotency and differentiation potential.

Is-15: β -Catenin Regulates Telomerase in Stem and Cancer Cells

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High telomerase activity is important for stem cells, aging, and cancer. Here, we report a molecular link between β -catenin and the expression of the telomerase subunit *Tert*. β -Catenin deficient (β -cat^{-/-}) embryonic stem (ES) cells have short telomeres; conversely ES cell expressing an activated form of β -catenin (β -cat^{ΔEx3/+}) have long telomeres. β -Catenin regulates *Tert* expression through the interaction with *Klf4*, a core component of the pluripotency transcriptional network. Binding of β -catenin to the *Tert* promoter is a general mechanism occurring in adult stem cells, in a mouse intestinal tumour model, and in human carcinoma cells. We uncover a novel link between the stem cell and oncogenic potential with β -catenin regulating *Tert* expression and, thereby, telomere length, which could be of critical importance in human regenerative therapy and cancer.

Is-16: Targeted Inactivation of Oncogenic Drivers of Cancer Originating in Adult Stem Cells during Muscle Regeneration

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The most prevalent types of cancer primarily affect tissues containing cells with increased proliferative potential often inferred by resident stem cells (SC) that enable regeneration of the respective tissue. Skeletal muscle regeneration is mediated by activation of rare quiescent muscle SCs that express *Pax7*. Recently it was shown that germline inactivation of *p53* in *mdx* mice undergoing chronic muscle regeneration develop rhabdomyosarcomas (RMS). However the cancer cell of origin and mechanisms of tumor formation under these settings have remained elusive. Coupling genetic lineage tracing and genomic analyses, we identified muscle SCs as a cellular origin of RMS and show that deactivation of muscle SC quiescence by regeneration is necessary to generate RMS upon SC specific loss of *p53*. Purification of lineage-traced tumor cells enabled identification of discrete genomic copy number amplifications that drive tumorigenesis including but not limited to *yap1*, *c-met*, *cdk4/os9* and *c-jun*. By reanalyzing human sequencing data including the TCGA PANCAN data set comprising more than 10,000 patients across a broad range of human cancers we discovered novel molecular subtypes of cancer. Importantly, targeted inactivation of identified oncogenes in individual primary tumor cells abolished tumor expansion. Our data indicate the dependence of individual tumors on distinct regulatory networks that originate from adult SCs and underscore the neces-

sity to provide means for personalized therapeutic interventions of cancer.

Is-17: Harnessing Functional Genomics to Identify Mechanisms of Stem Cell Dependent Tissue Regeneration

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Skeletal muscle stem cells (MuSC), also called satellite cells, are indispensable for maintenance and regeneration of adult skeletal muscles. Yet, a comprehensive picture of the regulatory events

controlling the fate of MuSC during regeneration is missing. We have integrated functional genomic approaches including genetic high-throughput loss-of-function screening to identify mechanisms regulating MuSC self-renewal and differentiation during skeletal muscle regeneration. In depth *in vivo* analysis revealed that MuSC-specific inactivation of epigenetic modifiers including the histone methyl transferase *prmt5* and the NuRD deacetylation complex prevents expansion of MuSCs, abolishes long-term SC maintenance and abrogates skeletal muscle regeneration by direct epigenetic regulation of discrete cell fate determinants.

Is-18: *In Vivo* Improved Wound Healing Using A Bilayer Skin Substitute Based on Silk Fibroin and Amniotic Membrane

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Treatment of full-thickness skin wounds with minimal scarring and complete restoration of native tissue properties still exists as a clinical challenge. We fabricated a bilayer skin substitute by coating human amniotic membrane with an electrospun nanofibrous silk fibroin layer to improve mechanical properties of the amniotic membrane (AM). Since this bilayer scaffold exhibited improved mechanical properties, surface hydrophilicity, and *in vitro* biocompatibility, *in vivo* biological behavior of this scaffold was investigated in murine full-thickness skin wound model. Donut-shaped silicon splints were utilized to prevent wound contraction in mouse skin and simulate re-epithelialization, which is the normal path of human wound healing. The skin regeneration using bilayer scaffold was compared with AM and untreated defect after 30 days. Tissue samples were taken from healed wound areas and studied by means of histopathological evaluation and immunohistochemical staining, to visualize involucrin (IVL), P63, Collagen I, CD31, and VEGF. In addition, mRNA expression of IVL, P63, interleukin-6 (IL-

6) and cyclooxygenase-2 (COX-2) was studied. Based on the obtained data, the application of bilayer scaffold resulted in the best epidermal and dermal regeneration, demonstrated by histopathological examination and molecular analyses. The mRNA expression levels of inflammatory markers (IL-6 and COX-2) in regenerative tissues by bilayer scaffold were down-regulated and expression pattern of keratinocyte markers including IVL and P63 markers at both mRNA and protein levels in bilayer scaffold group was more similar to native tissue in comparison to AM and no-treatment groups. There was no significant difference in expression level of Collagen I, CD31, and VEGF among different groups. Based on animal study results, preliminary evaluation of wound healing using this construct was done in 15 patients with foot ulcer after signing informed consent. The data showed safety and efficacy of the scaffold as well as wound area was significantly decreased from the beginning of treatment to the end of the first four weeks. Studies done in different phases were approved by Medical Ethic Committee of Avicenna Research Institute (ACECR, Tehran, Iran) and registered in Iranian Registry of Clinical Trials. Conclusively, these promising results suggest that this bilayer scaffold can represent a potential substitute for skin regeneration application and serve as supporting evidence for proceeding to major clinical phase.

Keywords: Skin Tissue Engineering, Bilayer Scaffold, Human Amniotic Membrane, Silk Fibroin, Wound Healing

Is-19: Nanotechnology Approaches in The Design of Hybrid Constructs for Regenerative Medicine

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In Regenerative Medicine, Tissue Engineering has been integrating principles of engineering, chemistry, materials science, biology and health sciences in order to develop regenerative-based therapeutic strategies combining stem cells and biomaterials. The development of hybrid devices for tissue engineering are often inspired by the composition and complexity of native tissues. At the lowest level of such organization, one should select the adequate biomaterials to be used as the building block of the structure that will support cells and control their behaviour towards the production of new tissue. Nanostructured multilayered films have been often fabricated using the layer-by-layer technology, where consecutive layers of macromolecules are well stabilized by electrostatic interactions or other weak forces. Such multilayered could be then integrated in more complex porous macroscopic devices, often exhibiting a multi-scale organization. Using adequate templates, non-flat multilayers can be fabricated with tuned compositions along the build-up assembly, including patterned membranes or porous devices. This enables the production of very well controlled multifunctional and structural devices using mild processing conditions that could be useful in biomedicine, including in tissue engineering. In particular, we have been interested in developing more complex/hierarchical porous structures using natural-based polymers that could fulfil specific requirements in such kind of applications. Methodologies developed in our group will be exemplified, permitting the production of (i) 3-dimensional (open) porous nanostructured scaffolds for tissue engineering, enabling the support of cells, by combining LbL and rapid prototyping

technology; and (ii) free-standing films featuring patterns to control cell orientation or micro-wells to provide local three-dimensional environments to the cells.

Is-20: Instructive Natural-Based Hydrogels as Platforms for Stem Cell Cultures for Tissue Engineering Applications

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The spatiotemporal organization of the stem cell niche provides valuable cues for the development of biomimetic environments that could have potential to stimulate the regenerative process. *In vitro* such highly hydrated 3D environments can be partially recreated using hydrogels. We propose the use of natural-based biomaterials to produce hydrogels able to encapsulate different cell types. In fact, due to their hydrophilic nature and richness in chemically active groups, such polymers can be used to produce a variety of crosslinked structures fabricated using aqueous-based or other environmental-favourable procedures. Examples are shown on such hydrogels obtained with distinct shapes, internal organization and sizes. Such biomaterials encapsulating mesenchymal stem cells may be used as implantable devices to regenerate tissues. Alternatively, in order to avoid diffusion limitation of nutrients to the cells location sites, hydrogel particles may be used to support cellular organization over their surface, acting as cells supports for injectable scaffolds. By decorating the surface with antibodies these particles are able to recruit specific cell populations, enhancing the therapeutic potential of such system. Polysaccharides may be also used to coat liquefied capsules that may entrap viable cells, using the layer-by-layer technology. The presence of solid microparticles inside such capsules offers adequate surface area for adherent cell attachment increasing the biological performance of these hierarchical systems, while maintain both permeability and injectability. The liquid environment allows for a free-organization in the space of the cells towards the formation of new microtissues. The compartmentalization of distinct cell types (including mesenchymal stem cells and endothelial cells) may enhance the osteogenic capability of this system, that could be useful in bone tissue engineering applications.

Is-21: Development of Functional Heart Ventricle Using Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

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Cardiovascular diseases (such as myocardial infarction and coronary heart disease) are reported as the most pernicious ones in the world. Current animal models cannot be reliable model for human heart performance due to noticeable differences in genetics, disease etiology and physiology, which limit their

utility. Moreover, laboratory studies were not able to evaluate volumetric performance of the heart. In this study, we aimed to fabricate a multilayer three dimensional ventricular construct similar to anisotropic orientation and function of native myocardium. This smallest functional target has thickness of 1.1 mm. Scaffolds with 11 layers, thickness of 0.1 mm and length of 1 cm for each layer were designed to resemble the model. Cardiomyocytes were autonomously self-assembled into architecture mimicking the native leftventricle. Scaffold properties were optimized to achieve high cellular attachment and proper orientation on the scaffold. A polydimethylsiloxane bioreactor was designed and fabricated to culture cell-laden scaffold. The cell-laden scaffold was then wrapped around a printed cone and cultivated for 7 days. The cell viability, alignment and contractility were evaluated for designed ventricular structure. We expect that multi-layer cardiomyocytes with similar orientation should be capable of synchronously twisting and contracting as the native left ventricle. The proposed research not only addresses urgent health problems in the world, but also helps develop a novel technology for one of the most highly demanded medical device markets. This artificial left ventricle would be an ideal platform for drug testing and pave the way for developing more complex heart chambers.

Keywords: hiPSC-Derived Cardiomyocytes, Left-Ventricle, Cardiac Tissue Engineering, *In Vitro* Model

Is-22: iPSC-Based Drug Discovery of Neurological Mitochondrial DNA Diseases

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Background: Mutations in mitochondrial DNA (mtDNA) cause diseases typically affecting the nervous system and for which no effective treatment exists. It has been difficult to develop animal models of mitochondrial diseases due to challenges of engineering mtDNA. Existing cellular models lack the metabolic features of neural cells and do not provide the patient-specific match between mitochondrial and nuclear genomes. Here, we propose to establish a novel system to carry our drug discovery experiments.

Materials and Methods: We generated neural progenitor cells (NPCs) from human induced pluripotent stem cells (iPSCs) and analyzed their genetic and metabolic properties. We also obtained NPCs from iPSCs derived from patients carrying pathogenic mtDNA mutations.

Results: We show that neural cells derived from human iPSCs display the correct functional and bioenergetics properties to investigate the neurological impairment associated with mitochondrial disorders. We used patient iPSC-derived neural cells carrying mutations in mtDNA genes to carry out a proof-of-principle high-throughput compound screening using FDA-approved compounds. We identify drugs that have the potential to be repositioned in the context of mtDNA diseases.

Conclusion: Patient iPSC-derived NPCs represent an effective model system in which carry out compound screenings. Our data pave the way to the identification of disease-modifying therapies for currently untreatable mtDNA disorders.

Is-23: Modeling Leigh Syndrome Using Patient-Derived Neural Cells

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Background: Leigh syndrome (LS) is a rare untreatable neurological disorder causing psychomotor regression and developmental abnormalities. LS is caused by mutations of nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) in more than 75 genes of the respiratory chain. LS research presently lacks good model systems for mechanistic studies, which hampers the discovery of therapies. We aimed to generate human induced pluripotent stem cells (iPSCs) from LS patients to dissect the disease mechanisms of LS

Materials and Methods: We generated iPSCs from LS patients carrying mutations in nDNA (in complex I and complex IV) and mtDNA (in complex V). We derived neural progenitor cells (NPCs) and neurons from patient iPSCs and analyzed their functional and bioenergetics properties.

Results: Neural progenitor cells (NPCs) and neurons from LS patients show bioenergetics impairment and functional defects including calcium dyshomeostasis and defective electrophysiological activity. The dysfunctions became stronger the longer the differentiation.

Conclusion: Our results suggest that LS mutations impair the bioenergetics of neural progenitors and post-mitotic neurons. Our findings represent the first model system of LS and provide insights into the mechanisms responsible for the developmental abnormalities and neurodegeneration observed in LS patients.

Is-24: Bioengineering of A Humanized Heart by Seeding of hiPSC-Derived Cardiovascular Progenitor Cells into Growth Factor-tethered Rat Heart Matrix

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Background: Millions of people worldwide suffer from cardiovascular diseases. Although current interventional and pharmacological approaches provide efficient therapies, curative treatment of end-stage heart failure is limited to heart transplantation. Bioengineering of whole hearts using human embryonic stem cells (hESCs)-derived cardiovascular progenitor cells (CPCs) and natural matrices is a promising approach to overcome organ donor shortage threatening millions of patients waiting for heart transplantation.

Materials and Methods: Here, we developed a novel strategy for generation of heart constructs by repopulating engineered decellularized rat hearts using hESCs-derived CPCs. We modified decellularization protocol to improve efficacy which was confirmed by multiple tests including DNA content analysis as well as biochemical studies. The decellularized hearts were recellularized by hESC-derived CPCs, which were generated in a scalable suspension bioreactor system. To improve CPCs proliferation and differentiation, we immobilized bFGF onto heart ECM prior to cell perfusion. Further optimization of seeding density and loading intervals allowed uniform recellularization of the heart scaffold. At day 12 post seeding, functional studies were performed on recellularized hearts. The beating rhythm was evaluated using a multielectrode array system.

Contraction motions were recorded using video microscopy and analyzed using a custom-made matlab macro. qRT-PCR and immunostaining was performed for cardiac specific markers. In-depth examination of the ultrastructure of seeded CPCs and CPC-derived cells were investigated by transmission electron microscopy (TEM).

Results: We demonstrated that perfusion-decellularization of whole heart allows the generation of a heart ECM scaffold with a perfusable vascular tree and intact 3D architecture, which acts as an efficient template to generate synchronously beating heart tissue. Comprehensive characterization of the decellularized heart matrix demonstrated preservation of complex ECM proteins, 3D spatial orientation and the micro-structure of native heart. Careful expansion of CPCs in a scalable stirred-suspension bioreactor combined with step-wise seeding (60 million cells in 3 steps of 20 million per 1.5 hour) onto decellularized hearts containing immobilized bFGF resulted in improved retention of CPCs and differentiation to cardiomyocytes, smooth muscle cells and endothelial cells as evaluated by immunohistochemistry and qRT-PCR. We observed spontaneous and synchronous contractions of humanized hearts after 12 days of perfusion as well as advanced alignment of myofilaments

Conclusion: While clinical implementation of engineered heart tissues is recently examining in clinical trials, the whole heart bioengineering science is evolving quickly in order to circumvent the heart transplantation obstacles in patients with end stage heart failure. Nevertheless, heart organogenesis via decellularization/recellularization is still facing multiple technological challenges before commercialization. Selection and large scale production of clinical grade starting cell ingredients, supplying heart natural scaffold, and more importantly improving cell repopulation procedure efficacy as well as the functionality of lab grown hearts are the main challenges which need to be addressed. Our study provides a robust platform for generation of artificial human hearts and resolves major bottlenecks hindering further development of this technology. Bioengineered hearts might soon find their way toward clinical application.

Keywords: Bioengineering, bFGF, Heart

Is-25: Revisiting LIF/GP130/JAK/STAT3 Signalling in Human Pluripotent Stem Cells

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Pluripotent stem cells (PSCs) can be derived and expanded in culture from the epiblast of the mammalian blastocyst. In mice, PSCs are called embryonic stem cells (ESCs). Inhibition of their differentiation and promotion of self-renewal are controlled by the LIF/GP130/JAK/STAT3 signalling pathway and do not require MAPK activity, which epitomizes the naïve (or ground) state of pluripotency. Although human PSCs are generated from the epiblast of the blastocyst similar to their rodent counterpart, they rely on FGF2 and Activin signalling for inhibition of differentiation, a property that epitomizes the primed state of pluripotency. All attempts to propagate human PSC lines using the culture conditions previously established to capture the naïve (or ground) state of pluripotency as defined in mice have been unsuccessful.

We sought to reactivate the LIF/GP130/JAK/STAT3 signalling pathway in human PSCs to know if this would sustain self-

renewal and inhibit differentiation as previously described in mice. Human PSCs fail to activate STAT3 target genes after LIF stimulation, LIF-independent GP130 dimerization, or GP130/JAK-independent activation of STAT3, indicating human PSCs are blind to STAT3 activity. However, combinations of the above, such as the concomitant activation of the GP130 receptor and overexpression of a GP130/JAK-independent STAT3, activate STAT3-target genes and inhibit differentiation. The transition from a FGF2- to a JAK/STAT3-dependent mode of self-renewal is accompanied by a transcriptome reconfiguration and by an alteration of cell-cycle parameters, consistent with a conversion to the naïve-like state of pluripotency. I propose that a synergy between STAT3 and one or several co-factors downstream of GP130 is necessary to sustain self-renewal of human PSCs.

Is-26: Naïve Pluripotency and Chimeric Competency in Rabbits and Non-Human Primates

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Pluripotent stem cells (PSCs) exist in two distinct state configurations, the naïve and primed states. These two states exhibit dramatic differences in their transcription and epigenetic profile, cell cycle regulation and energy production, which subsequently influence their characteristics and function. In rodents, only the naïve embryonic stem cells (ESCs) can colonise the blastocyst, contribute to the development of all tissue types, and generate germline chimeras. In rabbit and non-human primates, PSCs exist only in primed-like states and, consequently, they fail to incorporate into host blastocysts and participate in embryo development.

We aim to develop embryo colonization-competent PSCs in rabbits and rhesus monkey for developmental studies. Protocols originally developed to convert human PSCs to naïve-like pluripotency (i.e. cocktails of transcription factors, cytokines and kinase inhibitors) were systematically applied to rabbit and rhesus PSCs. The transcriptome of the converted cells was analysed and compared to that of the embryo. The capacity of the converted cells to colonise rabbit morulas and participate in embryo development was systematically assessed. Reprogramming protocols originally developed in human PSCs yielded very variable results in rabbit and rhesus monkey. Only some protocols yielded PSCs capable of extensive incorporation and survival into host embryos. A correlation was observed between colonisation competency and the yield of transcriptome reconfiguration. On the basis of these results, I will discuss the obstacles to overcome for successful generation of somatic chimeras in non-rodent species.

I-27: Modeling of Contextual Data Processing in The Visual Cortex

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This study focused on functional magnetic resonance imaging

(fMRI) experiments to explore, model and simulate possible link between contextual modulation and efficient macroscopic spatial response coding in the visual cortex. Specifically, we aimed to predict fMRI signal based on a computational neural network simulation. A sketch was provided, which covered practical steps to bridge the gap between mathematical modeling of single neuron responses to neuroimaging data with a mesoscopic biomimetic neural network. Then implemented the proposed biomimetic neural network to provide insight into data processing in cortical neural networks. In addition, showed that spiking frequency, entropy per spike and sparseness (as measures of network efficiency) are all associated with the natural contextual modulation.

Is-28: Myelinogenic Plasticity of Oligodendrocyte Precursor Cells and Role of Remyelination in Locomotor Recovery Following Contusive Spinal Cord Injury

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Spontaneous remyelination occurs after spinal cord injury (SCI), but the extent of myelin repair and identity of the cells responsible remain incompletely understood and contentious. We assessed the cellular origin of new myelin by fate mapping platelet-derived growth factor receptor α (PDGFR α), Olig2+, and P0+ cells following contusion SCI in mice. Oligodendrocyte precursor cells (OPCs; PDGFR α +) produced oligodendrocytes responsible for de novo ensheathment of at least 30% of myelinated spinal axons at injury epicenter 3 months after SCI, demonstrating that these resident cells are a major contributor to oligodendrocyte regeneration. OPCs also produced the majority of myelinating Schwann cells in the injured spinal cord; invasion of peripheral myelinating (P0+) Schwann cells made only a limited contribution. These findings reveal that PDGFR α + cells perform diverse roles in CNS repair, as multipotential progenitors that generate both classes of myelinating cells.

We subsequently assessed the necessity of myelin regulatory factor (Myrf) in remyelination after contusive SCI by deleting the gene from platelet-derived growth factor receptor alpha positive (PDGFR α -positive) oligodendrocyte progenitor cells (OPCs) in mice prior to SCI. While OPC proliferation and density were not altered by Myrf inducible knockout after SCI, the accumulation of new oligodendrocytes was largely prevented. This greatly inhibited myelin regeneration resulting in a 44% reduction in myelinated axons at the lesion epicenter. However, spontaneous locomotor recovery after SCI was not altered by remyelination failure. In controls with functional MYRF, locomotor recovery preceded the onset of substantial oligodendrocyte myelin regeneration. Collectively, these data demonstrate that MYRF expression in PDGFR α -positive cell derived oligodendrocytes is indispensable for oligodendrocyte myelin regeneration following contusive SCI but that remyelination is not required for spontaneous recovery of overground locomotion and stepping.

Is-29: Remyelination Failure Potentiates Axon Loss and Impairs Motor Function following Cuprizone Demyelination

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Multiple sclerosis (MS) is characterized by inflammatory demyelination, axonal degeneration and limited regeneration of myelin. Increasingly, axon loss has been linked to progressive disability, but its underlying mechanisms remain unclear. Oligodendrocytes support axonal survival in the healthy nervous system but there is currently little causative evidence demonstrating that remyelination is sufficient to increase axonal survival. Previously, we demonstrated that MYRF expression in new oligodendrocytes was essential for remyelination and a failure to express MYRF was linked with remyelination failure in MS. Here, we use an inducible Myrf knockout from OPCs (Myrf ICKO) following cuprizone/rapamycin intoxication to explicitly determine if remyelination is sufficient to preserve axons following demyelination. Myrf ICKO prevents the accumulation of new oligodendrocytes and results in a failure to remyelinate even seven weeks after cuprizone/rapamycin demyelination. Remyelination failure resulted in no overt changes in astrogliosis or inflammation but increased oxidative stress within the corpus callosum. A lack of oligodendrogenesis and remyelination left axons more prone to degeneration and subsequently impaired initial motor behavioural recovery. Therefore, improving remyelination is sufficient to ameliorate axon loss following inflammatory demyelination and will likely be an effective therapeutic strategy to attenuate axon loss in MS. This novel model will allow the testing of drug candidates for axonal protection after demyelination.

Is-30: From Stem Cells to Cerebral Cortex in A Dish: Mechanisms and Perspectives for Modeling Human Brain Diseases and Evolution

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The cerebral cortex consists of several hundreds of different types of neurons, organized into specific cortical layers and areas that display specific profiles of gene expression, morphology, excitability and connectivity. Pluripotent stem cells constitute a promising tool for the modelling of human brain development and diseases. Here, we will describe how corticogenesis *in vitro* from pluripotent stem cells can be used to identify the mechanisms underlying human neurodevelopmental diseases and to uncover novel links between cortical development and human brain evolution.

Is-31: Using Mouse-Human Chimeric Brain to Study Neuronal Development and Diseases

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The cerebral cortex is a highly complex structure that consists

of several hundreds of different types of neurons, organized into specific cortical layers and areas, that display specific profiles of gene expression, morphology, excitability and connectivity. Embryonic stem (ESC) and induced (iPSC) pluripotent stem cells constitute a promising tool for the modelling and treatment of human neural diseases.

Here, we will discuss how the transplantation of ESC/iPSC derived cortical neurons can lead to functional integration into developing and damaged cortical circuits, and how it can reveal novel mechanisms of human brain development and diseases, as well as open new perspectives for the repair of lesions of the damaged cerebral cortex.

Oral Presentations

Os-1: Injectable Tough Hydrogels for Engineering Load-Bearing Soft Tissues

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Injectable hydrogels are of great interest as tissue engineering scaffolds due to their biomimetic stiffness and ability for minimally invasive administration. However, the weak strength and brittle nature of conventional hydrogels have limited their use in load-bearing situations such as beating heart, contractile muscles or compressed cartilage. In our work, inspired from muscle structure, a dual crosslinking strategy is developed to answer the urgent need for self-recoverable, in situ forming tough hydrogels. Tuning the cooperative viscoelastic action of the ionic (calcium-carboxyl) and click (Diels-Alder) crosslinking within a single network of alginate provides a highly tough hydrogel with a set of interesting features: (i) in situ forming ability for minimally invasive injection or shaping by molding or extrusion, (ii) immediate self-recovery under cyclic loading, (iii) highly efficient and autonomous self-healing upon complete fracture, and (iv) capability for viable cell encapsulation and biomolecule conjugation. These features have not been totally met by the conventional tough hydrogels.

Os-2: Delivery of 1, 10-Phenanthroline and Cannabidiol Small Molecules Enhances Regeneration of Critical-Sized Radial Bone Defects in Rat

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Critical-sized bone defects are a major global health issue in orthopedics and cause mal-unions due to either an inadequate number of migrated progenitor cells into the defect site or their incomplete differentiation into osteogenic precursor cells. Although many attempts have been made to accelerate bone regeneration, an appropriate strategy to regenerate a new bone with optimum morphology and mechanical performance has not yet been introduced. The current study aims to develop a novel construct that contains cannabidiol (CBD) and phenanthroline (Phen)-loaded microsphere to recruit MSCs to the defect site and induce angiogenesis and regeneration of a critical size radial defect in a rat model. The scaffolds were fabricated by a freeze-drying technique and characterized in terms of morphology, structure, porosity and degradation rate. The effects of Phen and CBD delivery on cellular behaviours of viability, attachment, differentiation and angiogenesis were subsequently evaluated under *in vitro* condition. Radiology, histopathology and histomorphometry analysis of constructs were also performed 4 and 12 weeks post transplantation into the radial de-

fects. Physical characteristics of fabricated scaffolds confirmed the optimum biocompatibility, biodegradability and suitable mechanical properties of the scaffolds. *In vitro* and *in vivo* migration assays displayed a significant MSCs migration in the CBD treated groups. CBD-PLGA-G/nHAp, Phen-PLGA-G/nHAp and Phen-CBD-PLGA-G/nHAp significantly increased the osteogenic markers in comparison to the PLGA-G/nHAp and negative control groups. Real-time PCR analyses showed that expression level of angiogenic markers dramatically increased in scaffolds that contained Phen ($P < 0.05$). Computed tomography (CT) imaging combined with histomorphometry and IHC analysis showed enhanced bone formation and angiogenesis in all scaffolds compared to PLGA-G/nHAp group. Therefore, spongy scaffold that contained CBD and Phen and had MSCs recruitment and angiogenesis ability could be utilized to enhance bone regeneration of large-sized radial defects. **Keyword:** Bone Healing, Mesenchymal Stem Cell, Small Molecules, Scaffold, Radial Defects.

Os-3: Intra-Articular Knee Implantation of Autologous Bone Marrow-Derived Mesenchymal Stromal Cells in Rheumatoid Arthritis Patients with Knee Involvement: Results of A Randomized, Triple-Blind, Placebo-Controlled Phase 1/2 Clinical Trial

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Background: In this study, we intend to assess the safety and tolerability of intra-articular knee implantation of autologous bone marrow-derived mesenchymal stromal cells (MSCs) in patients with rheumatoid arthritis (RA) and to determine the preliminary clinical efficacy data in this population. The trial registration numbers are as follows: Royan Institute Ethics Committee: AC/91/1133; NCT01873625.

Materials and Methods: This single-center, randomized, triple-blind, placebo-controlled phase 1/2 clinical trial randomized RA patients with knee involvement to receive either an intra-articular knee implantation of 40 million autologous bone marrow-derived MSCs per joint or normal saline (placebo). Patients were followed up for 12 months to assess therapy outcomes.

Results: A total of 30 patients, 15 in the MSC group and 15 in the placebo group, enrolled in this study. There were no adverse effects reported after MSC administration or during follow-up. Patients who received MSCs had superior findings according to the Western Ontario and McMaster Universities Arthritis Index (WOMAC), visual analogue scale (VAS), time to jelling and pain-free walking distance. However, this improvement could not be significantly sustained beyond 12 months. The MSC group exhibited improved standing time ($P = 0.01$). In addition, the MSCs appeared to contribute to reductions in methotrexate and prednisolone use.

Conclusion: Intra-articular knee implantation of MSCs appeared to be safe and well tolerated. In addition, we observed a trend toward clinical efficacy. These results, in our opinion, have justified the need for further investigations over an ex-

tended assessment period with larger numbers of RA patients who have knee involvement.

Keywords: Bone Marrow, Mesenchymal Stromal Cells, Osteoarthritis, Rheumatoid Arthritis

Os-4: Hepatoprotective Effects of Intra-Splenic Injection of Extracellular Vesicles Generated from Human ES-MSCs on Chronic Liver Injury

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Hepato-protective effects of somatic tissue derived mesenchymal stromal cells (MSCs) against liver injuries have been widely observed in both experimental models and clinical trials. MSCs promote liver regeneration mostly through their paracrine effect following the secretion of extracellular vesicles (EVs). Human embryonic stem cell-derived MSCs (ES-MSCs) as an alternative source can overcome most challenges facing clinical application of somatic tissue derived-MSCs. The aim of this study is to illustrate biological properties of ES-MSCs and their generated EVs both *in vitro* and *in vivo* in Thioacetamide-induced chronic liver injury (CLI). We shows that ES-MSC can markedly inhibit the proliferation of peripheral blood mononuclear cells (PBMCs) in comparison to somatic tissue derived-bone marrow (BM)-MSCs and adipose (AD)-MSCs. Higher levels of anti-inflammatory cytokines (i.e. TGF- β and IL-10) and decreased IFN- γ were observed in the supernatant of ES-MSCs compared to other MSCs following MLR assay. Moreover, ES-MSC EVs also possess significant immunosuppression feature compared to other counterparts *in vitro*.

ES-MSC EVs exerted functional properties comparable to their parental cells and reversed fibrosis in TAA-induced chronic liver injury. ES-MSC EVs exhibited reduction of collagen density, necrosis, caspase density, portal vein diameter (PVD) and resolution of fibrosis. In addition, the results indicated down-regulation of major contributors to fibrosis (*Coll1a*, *aSMA* and *TIMP1*), pro-apoptotic gene (*BAX*) and pro-inflammatory cytokines (*TNF α* and *IL-2*) and upregulation of collagenase (*MMP9* and *MMP13*), anti-apoptotic gene (*BCL2*) and anti-inflammatory cytokines (*TGF- β 1* and *IL-10*) following intra-splenic injection of both ES-MSC and ES-MSC EVs in Thioacetamide-induced CLI.

In conclusion, these finding suggest that ES-MSCs and ES-MSC EVs as the next generation cell-free product can promote the recovery of liver injuries. Administration of allogeneic ES-MSC EVs as the next generation cell-free product may represent a novel therapeutic strategy for liver regeneration and it may considered as an alternative approach for stem cell based therapy for chronic liver injuries.

Keywords: Mesenchymal Stromal Cells, Extracellular Vesicles, Chronic Liver Injury

Os-5: Contribution of MicroRNAs to The Regulation of Ground State Pluripotency

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Embryonic stem cells (ESCs) are capable of unlimited self-renewal and multi-lineage differentiation into ectoderm, mesoderm, and endoderm derivatives. Because of these two key features, ESCs have numerous applications in studying embryogenesis, drug screening, disease modeling, and cell therapy. Self-renewal and multi-lineage differentiation potential of ESCs is orchestrated by an organized network of regulatory molecules including transcription factors and non-coding RNAs such as microRNAs. We show that microRNAs are differentially expressed in ground state ESCs compared to serum ESCs. We find that the imprinted *Dlk1-Dio3* locus expresses the majority of ground state-specific microRNAs, while the majority of serum ESC-associated microRNAs are encoded by the imprinted *Sfmbt2* locus. We further report that ground state microRNAs embedded in the *Dlk1-Dio3* locus (*miR-541-5p*, *miR-410-3p*, and *miR-381-3p*) promote pluripotency via inhibition of multi-lineage differentiation and stimulation of self-renewal. We also determine, for the first time, the global expression patterns of microRNAs over the course of ESC derivation from blastocysts and find that microRNAs are dynamically expressed and functionally important during this process. Taken together, we show that microRNAs are differentially expressed in different pluripotency contexts and during ESC generation, and that ground state microRNAs promote pluripotency by stimulating diverse aspects of self-renewal and inhibiting differentiation.

Keywords: *Dlk1-Dio3* Locus, Ground State Pluripotency, MicroRNA, Small RNA Sequencing, Differentiation

Os-6: Adipose Tissue- Derived Mesenchymal Stem Cells in Peritoneal Dialysis Patients

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Peritoneal dialysis (PD) is one of useful modalities whenever a patient reaches end stage renal diseases (ESRD). Worldwide PD penetration rate is 11% while in Iran is 4.1%. This modality is associated with some complications. Around 30-50 % of all patients who are on PD for 6 years, experience ultra-filtration fail-

ure (UFF). UFF is associated with peritoneal membrane thickness, inflammation and fibrosis which has no specific treatment. Moreover, UFF may ends in Encapsulation peritoneal sclerosis (EPS) with high mortality rate. In a retrospective study, we showed that longer time being on PD, younger age, and higher UFF duration are risk factors for peritoneal sclerosis.

Following promising results of mesenchymal stem cell (MSC) transplantation in PD models in recent decade, this method is taken into consideration in clinical trials. Experiments reported that MSC in PD models attenuated submesothelial thickness, inflammation, angiogenesis and fibrosis as well as improved ultra-filtration volume, glucose uptake, glucose mass transfer and solute transport. Taken together we hypothesized that MSC could induces anti-fibrosis, immunomodulation and anti-apoptosis effects in these settings. Therefore, we designed the first in human clinical trial of MSC in PD.

In this open label, non-randomized, phase I clinical trial, we infused autologous adipose derived MSC in 9 eligible patients and followed them for 6 months. Ten patients were regarded as control group. The primary endpoint was safety and the secondary endpoint was changes in membrane function.

14 Minor adverse events (AE) were recorded in MSC group which were subsided by itself or by supportive therapy. No serious adverse events or adverse events related to cell infusion were recorded. There was a significant reduction in Dialysate-to-plasma ratio (D/P) of creatinine in MSC group (0.77 to 0.73 P=0.02).

In summary, this trial showed safety, feasibility and tolerability of autologous MSC in UFF patients which could be a foundation for efficacy assessment by a RCT.

Keywords: Peritoneal Dialysis, Peritoneal Fibrosis, Ultrafiltration Failure, Adipose Tissue Mesenchymal Stem/Stromal Cell

Os-7: Two Nuclear Receptors NR5A2 and Rary Induce Human Naïve Pluripotent State

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Embryonic stem cells (ESCs) display two distinct states of pluripotency, naïve and primed, between which naïve state is in the topmost point of the developmental landscape. Accordingly, recent studies have focused on achieving naïve state in human ESCs, but functionality of the resulted naïve cells remains unclear. Here we report that transient activation of two nuclear receptors LRH-1 and RAR γ (2a) boosts impact of 2iL on inducing human naïve state during three different procedures including reprogramming human fibroblast to induced pluripotent stem cells, conversion of pre-existing human primed ESCs and direct

derivation of hESCs from embryo. 2a2iL-induced naïve cells unveiled several naïve specific criteria. Particularly these cells could contribute in mouse embryo and form interspecies chimera. Furthermore, a set of ligands including FGF4, NODAL, WNT3 and GDF3 were expressed in 2a2iL-induced naïve cells which is shared with the epiblast of human blastocyst. Therefore, these cells can be accounted as equivalent to preimplantation epiblast of human embryo. Mechanistically, 2a2iL naïve cells depend on TGF β pathway in both maintenance and derivation. Based on our findings, activation of TGF β pathway can be considered as a downstream target of 2a. We also showed that preconditioning TGF β along with 2iL is sufficient to interconversion of primed into naïve state. This study presents an approach for achieving functional human naïve pluripotent cells in a short period of time.

Keywords: Human Embryonic Stem Cells, Naïve Pluripotent State, TGF β Signaling Pathway

Os-8: Development of Pancreatic Organoids within A Bio-Engineered Device

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Pancreas development leads to beta cell formation via signaling interactions between specific cells and their adjacent tissues. Co-culturing mesenchymal stem cells (MSCs) and endothelial cells (ECs) with organ-specific progenitor cells has been recently shown to form a three-dimensional (3D) *in vitro* structures called organoids with near-physiological 3D architecture. In current study, we sought to examine human embryonic stem cells (hESC) as the exclusive source to derive all components of pancreatic organoids because such organoids can provide a valuable tool for studying the beta cell development and discovering new therapeutics for diabetes.

Using different stepwise protocols, the hESCs were differentiated into three cell types; pancreatic progenitor cell (PPC), MSCs and ECs. Once the true identity of these differentiated cell types were confirmed through cell specific markers, the ability of co-culturing these cell types for organoid production was tested with 4 different cell ratios on two distinct extracellular matrices, namely agarose and matrigel. Furthermore, the pancreatic organoid like structures (OLS) were transplanted in Nude mice and the level of human insulin and c-peptide in their serum was monitored for a period of three months. At the end, the grafts were removed and histologically analyzed.

The hESC derived PPCs (ES-PPC) were 82 \pm 6.4% PDX1+ with high levels of PTF1A and PAX6 which are expressed in earlier stages of pancreatic development. The hESC derived MSC (ES-MSC) showed high expression of CD markers (CD106 [87%], CD90 [97%], CD105 [96%], CD73 [95%] and CD44 [94%]) and were able to efficiently produce adipocytes and osteocytes. The hESC derived ECs (ES-EC) expressed high levels of CD144 and CD31 and also functionally expressed low

density lipoprotein receptors and showed angiogenic activity. Subsequently, four co-cultures with combination of three hESC derived cell types of ES-PPC, ES-MSC and ES-EC with ratios of 10:7:2, 10:7:3, 10:5:3 and 10:5:5, respectively, were prepared and tested. While only the “10:5:5 ratio” led to organoid-like structures (OLS) development after 48 hours on matrigel platform, no obvious organoid structures were observed in any of the four tested ratios on agarose platform even after applying forced aggregation method. Examination of cells inside of the generated OLS by immunostaining was performed and $41.71 \pm 1.37\%$ of cells were detected to be PDX1+. Further gene expression analysis showed the same patterns for PPC-specific markers for both OLS and ES-PPC. When transplanted in Nude mice, OLS resulted in vascularized grafts with $38.5 \pm 5\%$ insulin+ cells. Furthermore, human c-peptide and insulin secretion was also detected in the serum after 4 weeks.

Although improvements are needed to define the optimal protocol for complex vascularized and functional generation of pancreas organ, it can be concluded that this co-culture principle can provide a powerful system to study human pancreas development biology and disease modeling.

Keywords: Human Embryonic Stem Cells, Diabetes, Organoids, Beta Cell Differentiation, Pancreatic Progenitors.

Os-9: Augmented BMP4 Signaling Plays A Critical Role in Self-renewal of Mouse Embryonic Stem Cells

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Efficient control of the self-renewal and pluripotency maintenance of embryonic stem cell (ESC) is a prerequisite for translating stem cell technologies to clinical applications. 2i and R2i culture conditions are considered to maintain the pluripotency of mouse embryonic stem cells. In this study, we investigated the cell signaling and mechanism of the cells grown under 2i and R2i conditions. Previous high-throughput analysis indicated that BMP-related signaling mediators were upregulated in R2i versus 2i. To clarify the role of this pathway under pluripotent state, using functional pathway analysis we showed that the R2i cells were adversely affected and dead after treatment with BMP4 signaling inhibitors (Noggin and Dorsomorphin), while the proliferation and expression of pluripotency marker genes in 80% of 2i-grown cells decreased and the rest of the population showed resistance to this treatment. By the knockdown of downstream target genes (*Smad1,5,8, Id1 and Id2*) we showed that the inhibition of canonical pathway of BMP4 signaling has no effect on the survival of the cells, while inhibition of JNK as a target of non-canonical pathway of BMP caused a significant decrease in both of 2i- and R2i-grown cells. About 20% of 2i-

cultured cells showed resistance and survived against BMPi. We speculated that 2i is a heterogeneous population. To prove this hypothesis, 2i cells were cultured under BMPi up to five passages, to sorted resistance population in the presence of inhibitors which are known as 2i-sorted BMPi. We showed that 2i-sorted BMPi not only are pluripotent population, but also in comparison with 2i shows a higher potential in the expression of pluripotency marker genes. In conclusion, we revealed that inhibition of BMP4 signaling pathways, leading to death in R2i- and part of 2i-grown cells. By this treatment we could sort a pluripotent population of 2i which showed resistance against BMPi.

Keywords: Mouse Embryonic Stem Cell, Pluripotency, 2i, R2i, BMP4 Signaling Pathway

Os-10: Chemically Defined Culture Condition for Expansion and Maintenance of Human Pluripotent Stem Cell-Derived Early Cardiovascular Progenitor Cells

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Cardiovascular progenitor cells (CPCs) are suggested to be invaluable cell sources for a wide range of applications including experimental and clinical studies. One of the earliest type of CPCs is cardiogenic mesodermal cells (CMCs) which can generate almost all major types of cardiovascular cells. In order to benefit from early CPCs, large-scale production of CMCs in an *in vitro* culture system is required. In this study, we have attempted to introduce a simple, defined, and reproducible culture system for expansion, maintenance, and storage of CMCs derived from human pluripotent stem cells (hPSC). Some signaling molecules were screened to develop an efficient chemically defined culture medium. Cultured CMCs expanded for more than 10 passages, retained their morphology, gene expression pattern, chromosomal stability, and *in vitro* differentiation propensity into major cardiac lineages and exhibited regenerative potential when transplanted into the infarcted rat myocardium. We have observed the engraftment of the self-renewed cells and lack of tumorigenicity after transplantation. This serum- and feeder-free culture system is capable of transformation to a carrier-free suspension culture, which is required for large-scale production of hPSC-derived CMCs. Taken together, our results provide a novel approach for self-renewal and maintenance of early CPCs, which is a fundamental step for commercialization, developmental, tissue engineering, and cell-based clinical studies.

Keywords: Human Pluripotent Stem Cells, Early Cardiovascular Progenitor Cells, Expansion, Defined Medium

Poster Presentations

Ps-1: Comparison of Skin Transcriptome between Cell Transplantation Responder and Non-Responder Vitiligo Patients

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Background: Vitiligo is a disease that appears as a mucocutaneous hypo or depigmented macules and/or patches. So far, an autologous transplantation of epidermal cell suspension has been used in the three phases of clinical trial (Phases 1, 2 and 3) in Royan Institute to treat vitiligo patients, which is a simple, safe and relatively efficient method. However, the response to treatment is still not complete. Although all patients were included in previous studies based on the same clinical criteria, 24% of the patients did not respond to the treatment after 24 months follow up. In this study, we plan to identify potentially effective genes in the difference in response between responder and non-responders patients through comparing the transcriptome of these two groups of patients, in order to find probable indicators that could predict the response to treatment before this type of cell therapy.

Materials and Methods: 9 vitiligo patients who had been treated with an autologous transplantation of epidermal cell suspension were included in the study. Before cell transfer, two 2.5 mm punch biopsy samples were attained from the treated vitiliginous areas. Total RNA was extracted from these samples and transferred to -80 Freezer. These patients were also clinically followed up for 6 months. After 6 months of treatment, 5 patients responded to the treatment successfully, but four patients were left unanswered. The extracted RNAs of all these patients were sent for RNA sequencing.

Results: Sequencing results showed that the expression of 376 genes was different between responders and non-responders vitiligo patients. Furthermore, 213 genes were up-regulated and 163 were down-regulated. Based on each of these genes literature review and pathways analysis conducted us five candidate genes, ATP6V0B, PAX8, THRA, SRI and C9. Respectively, these genes are involved in melanogenesis, thyroid hormone signaling and synthesis, resistance to apoptosis, and complement and coagulation cascades where they may play role in several autoimmune diseases. We found ATP6V0B, PAX8 and SRI up regulate and THRA and C9 down regulate in responders in comparison with non-responder patients. Although the role of these genes in vitiligo has not been studied in papers, based on our findings in this study, it is believed that these genes would be effective in pathogenesis and possibly responding to cell therapy in vitiligo patients.

Conclusion: Based on the finding of this study, these 5 genes are likely to be considered in prediction of response to cell

therapy, although to generalize these results, it is necessary to investigate these genes in a large number of vitiligo patients.

Keywords: Transcriptome, RNA Sequencing, Cell Therapy, Vitiligo, Response Prediction

Ps-2: A Bioinformatics Approach to Identify Mesenchymal Stem Cells Soluble Factors Regulating Retinal Pigmented Epithelium Cells Development

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Background: Human pluripotent stem cells provide a promising cell source for ocular cell replacement therapy, but lack standardized differentiation protocols, yet. We aimed to develop a defined cell culture system for efficient methods to derive retinal pigment epithelium (RPE) cells from human stem cells. In the embryo, retina develops from neuroectoderm via the optic vesicle, while the surrounding extraocular tissues are necessary for normal eye growth and differentiation. Extraocular mesenchyme patterns the optic vesicles during early eye development. In our experience, co-culture of mesenchymal stem cells (MSCs) from different parts of the body during differentiation of human embryonic stem cell-derived retinal pigment epithelial cells results in distinct differentiation efficiencies. More specifically, co-culture of MSCs from the head has a significantly higher efficiency enhancement than body-origin. This experimental observation suggests that mesenchymal from head may have secreted factors that play a direct role in the induction of RPE cells. In the present study, using a bioinformatics approach, we compared expression profiles of MSCs originating from human head and body, in order to find effective factors in RPE induction.

Materials and Methods: All samples were obtained from the NCBI GEO database, and we included only healthy and untreated adult donor samples. To counterbalance tissue-specific effects, we integrated MSCs from variant tissues in the body. Using R/Bioconductor, we performed batch effect removal, quality control, dimension reduction and visualization of the data.

Results: From 16393 genes present in all samples, we identified 76 genes that were significantly unregulated in head-derived mesenchymal cells. Then, we focused on the genes with extra-cellular functions. This narrowed down our candidates to 22 genes. By scrutinizing pathways and biological functions of these candidates, we selected WNT5B, FBN2, and FBLN1 as the final candidates, to be experimentally validated.

Conclusion: Altogether, these data indicate the promising role of bioinformatics analysis in enhancing experimental proce-

dures of cellular differentiation, for regenerative medicine applications.

Keywords: Mesenchymal Stem Cells, Retinal Pigment Epithelium, Bioinformatics, Development

Ps-3: Identification of Novel Antisense Long Non-Coding RNA in Insulin-Producing Cells Derived from Adipose Mesenchymal Stem Cells under Shh Pathway Manipulation

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Background: Recent advances in a transcriptome analysis of the mammalian genome indicate the transcription of a large number of long non-coding RNAs (lncRNA) that have various roles in physiology of beta cells. Our previous works have shown that early inhibition and re-activation of the Shh signaling pathway results in production of functional insulin-producing cells (IPC), however the effect of Shh pathway manipulation on antisense lncRNA transcripts in IPCs derived from adipose mesenchymal stem cells (ATDMSc) is unknown. The main purpose of our study was to evaluate the effect of Shh pathway manipulation on antisense noncoding RNA transcriptome of mice ATDMSc during the differentiation into IPC

Materials and Methods: The ATDMSc cells were isolated from the adipose tissue around the testis of male C57BL/6 mice after digestion with collagenase type I and sequential passage and their pluripotency confirmed by analysis of expression of surface markers (CD44, CD90, CD31 and CD45), and adipogenic and osteogenic differentiation. ATDMSc cells were differentiated into IPC using basic three-stage protocol. At day 3 of differentiation the inhibition of Shh pathway was done using 0.25 μ M cyclopamine and 64 ng/ml bFGF, and on the 11th day of differentiation, the re-activation was performed using 150 ng/ml recombinant Shh. The expression of genes associated with endocrine function of beta cells including Insulin, Isl-1, Maf-A, Nkx6.1, Nkx2.2, Pdx-1, Ngn3, and GLI were evaluated on day 14 of differentiation using real-time PCR. In order to evaluate the expression change of antisense lncRNA, a microarray reaction was performed using Array lncRNA microarray mouse v.3 technology. The distribution of different types of antisense lncRNA on each chromosome, the changes in their expression in the manipulated IPC cells compared to the control cells, and their genetic distances from loci associated with insulin secretion and beta cell transcription factors were evaluated

Results: Of the total 1183 antisense lncRNA, the expression of 46 genes was upregulated more than 5 logfold and expression of 13 genes was downregulated lower than 5 log fold in Shh manipulated cells compared to unmanipulated cells. Evaluation of the genetic distance of down or up regulated lncRNAs with genes associated with function of beta cells showed their lowest distance with eight genes including FoxA2, Pax-6, Isl-1,

PED/PEA-15, Glis3, STXBP1, SYT4 and Kcnj15.

Conclusion: Regarding the significant change in the expression of some antisense lncRNAs near the functional genes of insulin-producing cells under the influence of Shh pathway manipulation, we concluded that they may be used for development of novel differentiation protocols for production of IPC cells in future studies.

Keywords: Adipose-Derived Mesenchymal Stem Cells, Shh Pathway, Insulin-Producing Cell, Transcriptome, Long Non-coding RNA

Ps-4: Triple Therapy with Bone Marrow Stromal Cells, Triiodothyronine (T3) and Exercise Reduced Apoptosis in An Experimental Model of Stroke

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Background: Nowadays, in order to improve efficacy of stem cell therapy, combination stem cells with other interventions has been considered. The purpose of this study was to examine effect of Bone Marrow Stromal Cells (BMSCs) combined with T3 and exercise (EX) on apoptosis in a mice stroke model.

Materials and Methods: The middle cerebral artery was occluded by an intraluminal filament for 45 minutes and then reperfusion was allowed for 7 days. BMSCs were injected into cerebroventricular space 24 h after ischemia and T3 and mild exercise were administered for six consecutive days. Apoptotic cells were evaluated using TUNEL assay in day 7 after stroke.

Results: Monotherapies with stem cells or exercise and Combination therapies with BMSCs+T3, BMSCs+exercise and/or BMSCs+exercise+T3 reduced apoptotic cells ($P < 0.05$). There was significant difference between BMSCs+exercise+T3 group and other groups expect BMSCs+exercise.

Conclusion: Other findings show that combination exercise and thyroid hormone could increase impact of stem cells on stroke-induced damage. Further studies are needed to clarify the efficacy of this therapeutic approach in stroke patients.

Keywords: Bone Marrow Stromal Cells, Focal Cerebral Ischemia, Thyroid Hormone, Exercise, Mice

Ps-5: Neuroprotective Effect of Mesenchymal Stem Cells Derived from Human Embryonic Stem Cell -Conditioned Medium on Ischemic Stroke Model

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Background: Stem cell therapy is a promising approach for stroke. Although low survival rates and potential tumorigenicity of implanted cells could reduce the efficacy of the cell-based treatment, recent investigations have proven that the use of stem cell-conditioned medium (CM) may be a feasible approach to overcome these limitations. There are numerous cytokines and growth factors in the CM of various stem cells responsible for the paracrine protective effects of stem cells. The purpose of the present study was to assess the effect of the conditioned medium of mesenchymal stem cells derived from human embryonic stem cell on infarct volume and neurological functions in ischemic stroke model rats.

Materials and Methods: Ischemic stroke was induced by standard right middle cerebral artery occlusion method (MCAO) in the 8-week old wister male rats. Injection of the CM or DMEM (5 μ l) was respectively done into the left lateral ventricle of the treatment and control animals from one hour following the surgery to the second day after MCAO induction (three doses). Behavioral tests including the cylinder and modified neurological severity score (mNSS) were performed on 1, 3 and 7 days after the injury. Infarct volumes were evaluated on 3 and 7 days after the MCAO by the 2,3,5 triphenyltetrazolium chloride (TTC) staining method.

Results: Our results indicated that 90 min occlusion of the artery caused impairments in sensory-motor functions in the ischemic rats. Furthermore, i.c.v injection of the CM improved functional recovery in the treatment group after the stroke. Volume analysis revealed that animals subjected to treatment with CM had significantly smaller infarcts than controls.

Conclusion: In this study, the therapeutic effects of the CM against stroke were confirmed in an animal model.

Keywords: Mesenchymal Stem Cells, Conditioned Medium, Ischemic Stroke, Neurological Function, Infarct Volume

Ps-6: Human Bone Marrow Mesenchymal Stem Cells Derived Extracellular Vesicles *In Vitro* Characterization on Rat Islet Cells

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Background: Mesenchymal stem cells (MSCs) have been increasingly used in treatment of type 1 diabetes (T1D). Extracellular vesicles (EVs) have been recognized as a mediators of stem cells and guiding their regenerative effects. Since the actual fractions of EVs and its function has not been characterized thoroughly on diabetes, current study aimed to investigate characterization of human bone marrow mesenchymal stem cell-derived EVs (hBMSC-EVs) on normal and diabetic Pancreatic rat islet derived cells.

Materials and Methods: Microvesicles (Mics) and Exosomes (Exs) isolated from supernatant of dynamically expanded hBMSC by differential ultracentrifuge. EVs were measured for their protein content using a BCA Protein Assay and then

characterized by electron microscopy and the particle size was measured by dynamic light scattering (DLS). Islet isolation was performed on healthy rats then islets were digested with 0.25% Trypsin-EDTA. Islet cells seeded 30,000 cells/well at a 96 tissue culture plate. To induce diabetes in pancreatic islet cells, alloxan was prepared freshly at a final concentration of 1mmol/l and incubated 14 h. then islets in two normal and diabetes groups, treated by three distinct Mics/Exs doses (0, 10 and 100 μ g/ml) separately. we tested if cells could uptake hBMSC-EVs labeled with red fluorcent PKH26 to follow their functional assay on dispersed rat pancreatic islet cells. To evaluate The effect of hBMSC-derived EVs on single cell viability we assayed dispersed islet cells using fluorescein diacetate (FDA) and propidium iodide (PI) staining. To evaluate beta cells proliferation, islet cells were stained by immunofluorcent (IF) against Ki67 and insulin.

Results: We quantified that according to the amount of 36×10^6 hBMSC could produce approximately 1218 μ g exosomes and 1190 μ g microvesicle. DLS and electron microscopy also have been done for the collected EVs. Cells were plated at 30,000 cells/well and incubated with EVs at different concentration (0, 10, 100 μ g/ml) and the control (DPBS) for 48 h. co-culture of islet cells with PKH-26 labelled EVs, showed that EVs could be internalized by normal islet cells and also diabetic islets at 48 h. FDA- PI staining also showed the effect of hBMSC-EVs on viability of dispersed rat islet cells in normal and diabetic group. The results of immunofluorescence co-stain with insulin and Ki-67 showed proliferation rate of diabetic and normal islet cells.

Conclusion: The current study tries to figure out and characterize the function of two main fractions of EVs, microvesicles and exosomes, on diabetic and normal rat islet cells *in vitro*. In conclusion, our study shows that MSC-derived EVs have significant potential as an alternative to cell therapy for autoimmune diseases prevention.

Keywords: Diabetes, Mesenchymal Stem Cell, Extracellular Vesicles, Exosome, Microvesicle

Ps-7: Transfection Efficiency Evaluation of Retinal Progenitor Cells Using Cationic Lipid-Based Reagents

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Background: The retina is the significant neural tissue that localizes in the posterior layer of the eyeball. During development, retinal progenitor cells (RPCs) differentiate to different types of neural retinal cells. Although, recently, RPCs have been considered in many preclinical studies, its low transfection potential in genomic studies requires more investigations. For this purpose, in this study different lipid-based reagents (lipofectamine LTX, 2000 and 3000) were applied as gene carrier to optimize the transfection efficiency.

Materials and Methods: Human embryonic stem cell line (hESC), RH6, was obtained from Royan institute. Under appropriate culture condition using combination of Noggin, IWR

and IGF-1, RH6 cells were differentiated to RPCs. Next, gene expression profile of several eye field markers were characterized by immunocytochemistry (ICC) and quantitative real time PCR. The vector pEGFP-C1 (CLONTECH) containing the constitutive promoter of human CMV (driving the expression of EGFP gene) and SV40 polyadenylation signals; was used to allow visualization and analysis of transfected cells using lipofectamine LTX, 2000 and 3000. The transfection efficiency was analyzed with flow-cytometry and qRT-PCR.

Results: hESC derived-RPCs expressed eye field markers RAX, PAX6, LHX2 and SIX3; whereas the stemness markers such as NANOG and OCT4 were reduced in compared to hESCs. The results of cationic lipid-mediated transfection of RPCs showed the highest transfection efficiency with lipofectamin LTX, whereas lipofectamin 2000 resulted in very low efficiency and also toxicity.

Conclusion: Our data demonstrated that high yield of RPC could be obtained from hESCs. Moreover, RPCs showed the highest transfection potential with lipofectamin LTX in between different cationic reagents. These results could be used for genetic manipulation of RPC studies.

Keywords: Retinal Progenitor Cells, Transfection, Lipofectamin Reagent, pEGFP-C1

Ps-8: Dental Pulp Stem Cells Show Different Panel of Drug Resistant Genes Compared to Dental Pulp Fibroblasts

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Background: Previously, we have shown that dental pulp derived stem cells are more resistant to the cytotoxic effect of restorative dental materials as compared to dental pulp stem cells. Here, we aimed to find the genes involved in this resistance.

Materials and Methods: Based on the CD146 expression, magnetic cell sorting was done to purify DPFs and DPSCs from cultures of pulp derived cells. Colony forming assay, differentiation, and flowcytometry was performed to characterize the cells. the mRNA expression of 14 ABC transporters including ABCA2, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5-2, ABCC5-4, ABCC5-13, ABCC6, ABCC10, ABCC11, and ABCG2 genes were assessed in both CD146 positive and negative cells.

Results: Only CD146+ group of cells could differentiate into adipocytes and osteoblasts and only this group of cells could form typical colonies. Consequently, CD146+ cells were considered as stem cells and negative portion were considered as fibroblasts. All genes showed mRNA expression in both cells except for ABCB11 and ABCC11 genes. In CD146+ stem cells, ABCA2, ABCB1 and ABCC5-2 genes showed the higher expression (approximately 2-fold) whereas ABCC6, ABCC10 and ABCG2 genes had lower expression (about half of the other genes) of mRNA compared to the remaining genes.

Conclusion: Our results reveal that only that differential ex-

pression of ABC gene family may explain differential ability of stem cells to protect themselves against cytotoxic agents compared to fibroblasts.

Keywords: Stem Cell, Fibroblast, Dental Pulp, CD146, Drug Resistance Genes

Ps-9: Regulatory Networks in Breast Cancer Stem Cell Response to Selumetinib

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Background: The mitogen-activated protein kinase (MAPK) signaling pathway has a role in cell proliferation and survival. The mitogen-activated protein kinase (MEK) activity is vital for MAPK signaling. So, targeting of MEK in malignant cells have been done in many studies. Selumetinib drug is a non-ATP- competitive MEK inhibitor, has antitumor activity in variety of different tumor type. Selumetinib in combination with chemotherapy drugs inhibit tumor growth and induce apoptosis more effective. Recently, many studies have been shown that selumetinib inhibits cancer stem cell in breast cancer via affect MEK expression. According to these data, selumetinib can be useful for treatment of cancer and reduce tumorigenic activity.

Materials and Methods: In this study for getting different genes expression in MAPK pathways that were affected by selumetinib After 4h and 24 h, first we obtained the gene name list for this study. Then gene ontology analyses were conducted to find the most affected genes. Most important TFs were studied and using all these knowledges a core regulatory network was constructed for comparison.

Results: Our approach revealed many aspects of gene expression. We could correlate some of these differences to key regulatory elements TFs. Network analysis uncovered major differences in core regulatory network between these genes in varied pathways.

Conclusion: These analyses would be useful for clinical application. We have identified that different genes don't have the same expression. We hope that our analysis facilitates selection of the most appropriate genes for cancer therapy.

Keywords: Mitogen-Activated Protein Kinase (MAPK), Selumetinib, Breast Cancer Stem Cells

Ps-10: DNA Methylation Dynamic during Differentiation of iPSCs to Endoderm, Mesoderm, Ectoderm

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Background: Induced pluripotent stem cells (also known as iPSCs or iPSCs) are type of pluripotent stem cell that can be generated directly from differentiated tissue. Like that, differentiated cells can be reprogrammed into iPSCs with the over-expression of reprogramming factors such as: OCT4, KLF4, SOX2, cMYC, NANOG. Also, iPSCs have distinct molecular and functional properties and varied DNA Methylation exists in them. Thus, they have ability to differentiate into any type of tissue in body including: endoderm, mesoderm, and ectoderm.

To this reason, study of epigenetic variation in cells derived from iPSCs is important. However, this topic is studied poorly.

Materials and Methods: In this regard, first we have obtained the list of related genes. Then we have used Integrated Genome Browser (IGB) software (version 9.0.0) to find DMR in the 3 tissues, endoderm, mesoderm, and ectoderm. Next, DNA methylation and gene expression patterns were integrated to understand the impacts of methylation dynamics on differential gene expression.

Results: In this study, we investigated that varied DNA methylation in iPSCs leads to generating different tissues. We found some important genes endoderm, mesoderm, ectoderm and compare DNA methylation pattern to find out whether iPSCs make different methylation pattern.

Conclusion: In this study showed that some of gene have differential methylation pattern in in direct differentiation to three type of tissue that it helps us to choose key genes for make special tissues.

Keywords: Induced Pluripotent Stem Cells (iPSs), Differentiation, DNA Methylation

Ps-11: Bioinformatics Assessment of The Gene Network and Non-Coding Regulators of Inflammatory Pathway in Differentiation of Naive CD4 + T Cells to Th17 in Multiple Sclerosis and Type 2 Diabetes

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Background: Type 2 diabetes and multiple sclerosis are two conditions caused by various factors, including inflammation. Th17 cells play a significant role in the progression of inflammation. These cells, which are differentiate from naive CD4+ cells, may encounter inappropriate differentiation and proliferation due to various disorders in the genes and their regulators, which may lead to inflammatory symptoms. The expression of these genes can be regulated by non-coding RNAs. As a result of the disease, numerous changes can be occurred in the expression of long non-coding RNA and microRNA, which subsequently affects the expression of genes in the differentiation process. The result of this inappropriate change may cause diseases such as T2DM and MS.

Materials and Methods: Initially, microRNAs, long non-coding RNA, and their related genes were collected by textmining and from GEO database. Subsequently, several parameters were determined to select several thousand genes and non-coding RNAs using the Python programming language. The program output was evaluated and analyzed by several LncRNA and miR databases. R programming language was used for the final analysis. At the end, a network between selected genes and non-coding RNAs was visualized by Cytoscape software.

Results: Non-coding RNAs are considered as the vital regulators of biological processes. As a result of disruptive changes of these regulators, various pathways get disturbed. Due to the similarities between inflammation-induced Th17 differentiation in T2DM and MS, a significant network was created and it was

shown that among more than eight thousand non-coding RNA and dozens of genes which are involved in the differentiation pathway, 127 non-coding RNA and 19 genes play crucial roles in regulating this process.

Conclusion: Investigations of these interactions indicate a logical network between lncRNA, miR, and genes and it was shown that non-coding RNA could be considered as important biomarkers in T2DM and MS.

Keywords: Th17 Differentiation, MicroRNA, Long Non Coding RNA, Multiple Sclerosis, Type 2 Diabetes

Ps-12: Interferon Beta Ameliorates Amyloidosis and Increases Neurogenesis in The Hippocampus of Lentivirus-Induced Rat Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common type of dementia. The impairment of learning and memory and neuronal loss are characteristics of AD, which are associated with the overproduction of beta amyloid (A β) peptide in the cortex and hippocampus. In addition, studies suggest that dysfunctional neurogenesis in the hippocampus might also contribute to the neurodegenerative process and memory deficits observed in AD. It is also known that neuro-inflammation can lead to A β aggregates formation which limit the survival of newborn neurons in the dentate gyrus (DG). Therefore, stimulating neural stem cells (NSCs) to replace lost neurons is a promising approach for AD treatment. Interferon beta (IFN β) is the primary treatment used to combat inflammation and flare-ups in multiple sclerosis. IFN β was reported to have anti-inflammatory and anti-apoptotic properties. The current study aimed to examine whether intranasal (IN) IFN β treatment with high CNS bioavailability and minimal systemic side effects, could ameliorate memory impairment by decreasing amyloidosis and promoting neurogenesis in a rat model of AD.

Materials and Methods: Lentiviruses (LV) encoding human amyloid protein precursor (hAPP) bearing Swedish and Indiana mutations (LV-hAPP^{Sw/Ind}) (3 μ l; 109 TU/ml/site) were injected bilaterally in the hippocampus CA1 area of adult male rats. Cognitive function was assessed using Y-maze task on day 43 after injection. Furthermore, we evaluated amyloid precursor protein (APP), Ki-67 (a proliferating cell marker) and doublecortin (DCX; an immature progenitor cell marker) immunolabelling in the hippocampus. A thflavin S staining method is also used to identify A β plaque deposition levels. Therapeutic effects of IN IFN β delivery (1 μ g/kg, started from day 23 after injection and continued every other day for 14 total doses) were also investigated in AD animals.

Results: Our results showed that injection of LV-hAPP^{Sw/Ind} induced a significant decrease in the spontaneous alternation behavior compared with sham group, and this was accompanied by an increased number of APP positive cells and A β plaque deposition in the hippocampus of AD animals. IFN β treatment attenuated cognitive deficit induced by LV-hAPP^{Sw/Ind}. In addition, IFN β significantly decreased the number of APP positive

cells and A β plaque deposition in the hippocampus of AD animals. Interestingly, rats treated with IFN β showed significantly increased numbers of Ki67 and DCX positive cells in the DG area of hippocampus.

Conclusion: The results of this study indicate that IFN β treatment of AD-rats can enhance NSCs proliferation and neuronal differentiation by reduction of APP expression and A β plaque deposition which may finally lead to improvement of spatial cognitive function. Taken together, these results provide evidence that IFN β can potentially be used as a therapeutic agent in treatment of AD.

Keywords: Alzheimer Disease, Interferon Beta, Beta Amyloid, Neural Stem Cells, Memory

Ps-13: Differentiation of Rat Adipose-Derived Mesenchymal Stromal Cells toward Insulin-Producing Cells

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Background: Until recently, much effort has been directed toward finding novel stem cell sources and improving differentiation protocols to develop a promising therapeutic method for treating T1DM (Type 1 Diabetes Mellitus). In this study, we propose a novel three stage differentiation method for efficient differentiation of ATDMSCs to functional IPCs.

Materials and Methods: ATDMSCs were isolated from rat adipose tissue using collagenase type 1. ATDMSCs were differentiated into IPCs via a 14-day basic protocol using 1% insulin transferrin selenium (ITS) and 1% nicotinamide in Dulbecco's Modified Eagle's Medium. Insulin granule formation, glucose-stimulated insulin secretion and gene expression pattern related to the pancreatic endocrine development and function were analyzed in obtained IPCs (Insulin Producing Cells). The diabetic rats (n=10) were divided into two groups. One group (n=5) received undifferentiated ADMSCs; another group (n=5) was injected with differentiated IPCs. Then, 1×10^6 of the isolated cells were injected to the tail vein of the rats. Fasting blood glucose concentrations were measured once a week. At the end of the sixth week after transplantation, the blood insulin concentrations were determined.

Results: The obtained IPCs exhibited significant expression of MafA, Nkx2.2, Nkx6.1, Ngn3, insulin, and Isl1, and insulin secretion ability in response to glucose challenge. Rats that received the differentiated IPCs exhibited a higher ability to normalize blood glucose and insulin secretion when compared to control group.

Conclusion: The functional IPCs were successfully obtained from ATDMSCs using a three-step protocol. The potency of the differentiated IPCs for the secretion of insulin and glycemic control showed a significant increase compared to the un-dif-

ferentiated ATDMSCs *in vivo*. However the differentiated IPCs were unable to restoration of euglycemia and normal acute insulin response to glucose. Further examinations are required to improvement of the three stage differentiation protocol for more efficient cell therapy of diabetic patients in future.

Keywords: ATDMSCs, IPCs, T1DM, Stem Cells

Ps-14: Key MicroRNAs during Single-Cell Reprogramming of Cardiac Cells from Mouse Fibroblast in Early Stage of Induction

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Background: Generation of functional heart that work as a natural one is one of the field of regenerative medicine. Although, over the past decade, cellular replacement therapies for recovering injured heart has received considerable effort, have not accessed much success. Heterogeneity of cell is one of the most significant causes of failure. Therefore, using single cell RNA-Seq has overcome this limitation. Also, it seems that before doing any proceeding interest which consumes time and cost, it is useful to use bioinformatics approach to find regulatory factors like microRNAs. In this study, we have used bioinformatics approaches for single cell RNA-Seq datasets to find most important microRNAs and their target genes for post transcriptional gene silencing in early stage of direct conversion from fibroblast to cardiomyocytes with Mef2c, Gata4 and Tbx5 in early stage of induction.

Materials and Methods: Gene expression obtains from Gene expression omnibus of National center for biotechnology information for single cell RNA-Seq dataset. False discovery rate less than 0.05 and fold change more or less than 2 and -2, respectively was used as a cutoff for differentially expressed genes. Differentially expressed genes were submitted to miRwalk, the database for prediction and validation miRNA-gene interaction. We have used valid information with laboratory evidence for 3' and 5' untranslated region with p.value less than 0.05. Also, we have constructed gene regulatory network to find hub microRNAs and their targets.

Results: MicroRNA-gene interactions were identified for mouse cardiac reprogramming. These key microRNAs were identified for early stage of direct conversion, include seven and fourteen days from single-cell RNA-Seq datasets.

Conclusion: This finding could shed a light to better understanding the molecular mechanism, especially post transcriptional gene silencing under direct conversion of fibroblast in to cardiomyocyte according to eliminate cell heterogeneity. Also, to uncover post transcriptional gene silencing among early stage of induction, seven to fourteen days.

Keywords: MicroRNA, Network Analysis, Regenerative Medicine, Single-Cell RNA-Seq

Ps-15: Microfluidic Platform for Controlled Synthesis of Polysaccharides-Based Nanoparticles for Anticancer Drug Delivery

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Background: Nanotechnology has the potential to revolutionize cancer diagnosis and therapy. Various types of nanoparticles (NPs) have been used in delivering anticancer drugs to the site of action. Among the various approaches for synthesizing therapeutic nanocarriers, microfluidic systems techniques which were originally developed in the microelectronics industry, have emerged as a promising platform for designing advanced drug delivery systems with their capability for precise handling and transport of small liquid quantities. This is particularly important since the synthesis of biodegradable polymeric nanoparticles by bulk mixing and nanoprecipitation of drugs and biodegradable polymeric precursors typically lacks control over the mixing processes, which may compromise the properties of the resulting nanoparticles. Rapid and tunable mixing in microfluidics may allow for better control over the process of nanoprecipitation and also enable screening of various formulation conditions on a single platform by varying parameters such as flow rates, precursor composition, and mixing time. These techniques enable production of mono dispersed and multifunctional drug carriers with highly tunable physical and chemical properties to promote efficacy of drug transport, release, distribution, and elimination during the course of treatment.

Materials and Methods: In this study, we developed a T-shape microfluidic device to create hydrodynamically focused flow consists of two inlets, one for tripolyphosphate (TPP) at basic pH (pH>7), one for chitosan-drug at acidic pH (pH <6), and one outlet for the fabricated nanoparticles. Chitosan as a natural polymer has some unique properties which highlight it as an excellent carrier for anti cancer drugs. Microfluidic devices were fabricated with poly (dimethylsiloxane) (PDMS) using a standard micromolding process. To make the master molds, silicon wafers were spincoated with SU-8 2050 photocurable epoxy to a thickness of 60 μ m. The mixing channel was 150 μ m wide, 60 μ m high and 1 cm long. Stability and hydrodynamically focused of the flow at different flow rates was verified using inverted microscope. Chitosan has the ability of gelation spontaneously, in contact with multivalent polyanions due to the formation of inter- and intramolecular cross-linkage mediated by the polyanions. The mixing time is controlled on the millisecond scale for the ionic crosslinking reaction by changing the flow ratio. Size of on chip synthesized nanoparticles was determined by DLS method.

Results: Unfortunately, nanoparticle self-assembly with simple bulk mixing produces extremely polydisperse chitosan nanoparticles, which in turn exhibit a wide range of mechanical and chemical properties. However, we can create small and monodisperse nanoparticles with microfluidic synthesis by creating a narrowly defined mixing regime via hydrodynamic flow focusing. A critical parameter in determining nanoparticle size is the mixing time. The mixing time used for self-assembly is kept in the millisecond range via changing the flow ratio of chitosan-drug to TPP streams from 0.05 to 0.1(ml/min). We produce kinetically locked nanoparticles, which prevents their further growth, and keeps their final size smaller and more monodisperse than any bulk method. Chitosan-drug nanoparticle morphology trends found through dynamic light scattering analysis were confirmed with Scanning electron microscope (SEM). The diameter of the nanoparticles increases with an increase in flow ratio. Moreover, the polydispersity index (PI) for the microfluidic nanoparticles was found to be remarkably lower (PI<0.2) than bulk synthesized particles.

Conclusion: In summary, we have developed a microfluidic-

assisted fabrication of complex chitosan- drug showing highly efficient encapsulation capacity of hydrophobic drugs. The monodisperse chitosan-drug in the range of 100-300 nm, ideal sizes for drug delivery,

Keywords: Nanotechnology, Drug Delivery, Microfluidic System, Controlled Synthesis, Nanoprecipitation

Ps-16: Effect of C-KIT+ Bone Marrow Stem Cells Co-Culture on Angiogenic Factors of Cardiomyocytes Derived Adult Rat Heart

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Background: Heart failure remains a leading cause of mortality in the World. Reduction in myocyte number and cardiac pump dysfunction are marked in many of the heart failure such as myocardial infarction. So, find a method to recover cardiomyocyte function after myocardial injury or to increase the number of cardiomyocytes, it has been a long-term goal. Use of stem cells which can be transplanted into the infarcted myocardium that can repair and regenerate the lost tissue and improve cardiac function, as an attractive method has been reported. The use of C-kit+ cardiac stem cells (CSCs) as an appropriate candidate cell for future cardiac regenerative medicine strategies is promising. C-kit+ CSCs have been extensively examined, consistently exhibit several *in vitro* characteristics that define a "stem cell", being clonogenic, self-renewing and multipotent potential. But the cellular mechanisms of C-kit CSCs have yet to be reported. The aim of this study is to determine the effect of C-kit+ CSCs co-culture on angiogenic factors of cardiomyocytes derived adult rat heart via VEGF signaling pathway.

Materials and Methods: C-kit+ stem cells were isolated from bone marrow of adult *rattus norvegicus* by magnetic cell sorting (MACS) (Miltenyi), and the purity of the isolation was assessed by FACS analysis. Subsequently, cardiomyocytes were isolated from adult rat heart by enzymatic digestion and both cell types were co-cultured with each other for 48 hours. In the following, cardiomyocytes were collected; quantitative Real-time PCR and western blotting were used for the detection of gene expression of GATA4 and TBX5 and protein expression of VEGF, respectively.

Results: The results of this study showed that the expression of GATA4 and TBX5 genes and VEGF protein was significantly increased in the cardiomyocytes co-cultured by the C-kit+ cells as compared by control group.

Conclusion: In this experiment, it can be concluded that changes induced in the gene and protein expression level in cardiomyocyte derived adult rat heart co-cultured with C-kit+ bone marrow stem cells via VEGF signaling pathway.

Keywords: C-kit+ Bone Marrow Stem Cells, Magnetic Cell Sorting, VEGF Signaling Pathway

Ps-17: Identification of Transcription Factors in Prostate Cancer Stem Cell Line via miRNA-Mediated Silencing

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Background: Prostate cancer (PCa) is a common disease in men, who are more than 50 years old. This cancer has poor prognosis. Therefore, identification of treatment options can be important. In keeping with many studies, minority of tumor cells population cancer stem cells (CSCs) are response to self-renewal, differentiation, tumor progression. So, targeting CSCs reduces tumor growth. MicroRNAs (miRNAs) are a class of small non-coding RNAs molecule. miRNAs found in plants, animals and some viruses and play a role in regulation of gene expression. Although, there aren't expression of microRNAs changes in human cancer stem cells but effect of microRNAs on PCa is unknown. Have been shown that Silencing of the miR-424 leads to degradation transcription factors in prostate cancers. Thus, we can use them for treatment of cancer stem cells in prostate cancer by focusing on the genes involved in the disease.

Materials and Methods: To better interpret the role of miR-424, we have obtained gene symbol the cells in which miR-424 is Silenced and control samples in prostate cancer stem cells. In this study first we got name of these gens by David tool. In the next step by using Enricher we got transcription factors (TFs) for genes, finely we draw network by Gephi software.

Results: By these analyses we have identified the main TFs and using network we have showed integration between these TFs and the target genes.

Conclusion: These analyses would be useful for clinical application. We have identified role of miR-424 on Prostate cancer TFs.

Keywords: Prostate Cancer, Network Analysis, MicroRNAs, Cancer Stem Cells

Ps-18: Epigenetic Marks Comparison between Neural Cells-Derived from iPSCs and ESs

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Background: The epigenetic memory of a cell is a set of modifications to the cell deoxyribonucleic acid (DNA) that do not alter the DNA sequence. These modifications can alter gene expression. Induced pluripotent stem cells (iPSCs) have been generated from various somatic cell types. Many study have been suggested that iPSCs from different cell sources have distinct molecular and functional properties including self-renew differentiate into any specialized cell of the body. Although, iPSCs can make almost varied cells, there are concerns about variation in the differentiation capacity of these pluripotent cells compared to ESCs. It would be useful that we detect the major sources of variation for their use in clinical applications and disease modeling. To uncover these variations, we have investigated histone methylation marker during direct differentiation of iPSCs and ESCs into Neural cells.

Materials and Methods: We have used data set to address the issue with differential capacity among ES and iPSCs. We obtained differentially expressed (DE) genes. Next, methylation pattern of promoter regions of these DE genes was assessed. Then, differentially methylated regions (DMR) that are in-

involved in defining differential capacity were extracted.

Results: Our analysis shows that expression patterns of some genes are different. These data identified DNA methylation as an essential epigenetic mechanism and determines stem cell functions. Additionally, affected DMR regions causing differential gene expression pattern showed promising results.

Conclusion: Here we demonstrate that ESCs and iPSCs have an epigenetic memory of their tissue of origin and it had led to different gene expression patterns. These analyses revealed the principal differences between iPSCs and ESCs. We hope these analyses would be beneficial for applications in disease modeling or treatment.

Keywords: Induced Pluripotent Stem Cells, Embryonic Stem Cells, Differentially Methylated Regions

Ps-19: Gene Regulatory Network of Mesenchymal Stem Cells Response to Rosiglitazone

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Background: Originating stem cells are divided into two major categories of embryonic and adult stem cells. One of the most important adult stem cells are mesenchymal stem cells. Mesenchymal stem cells were first isolated from the bone marrow, but subsequent studies showed that some tissues of the body also contain mesenchymal stem cells. Thiazolidinediones (TZD) are widely prescribed for the treatment of Type 2 diabetes. osiglitazone (RGZ), one of the TZD, induced adiposity in mesenchymal stem cells. Since adiposity cells play an important role in energy homeostasis, differentiation in to adipocytes is an important parameter in adipogenic studies. The treatment of mesenchymal stem cells with an adipogenic stimulant rosiglitazone, induces adipogenic differentiation. Rosiglitazone can be used for cell therapies.

Materials and Methods: To find the role of rosiglitazone on MSCs, first we get different genes expression of treated cells in three times that were affected by rosiglitazone, first we obtained the gene name list involved in ever time, then apply threshold less 0.05. We obtained miRNAs for 72h,24h and2h, and finally draw network by Gephi software.

Results: By these analyses we have identified the several miRNAs and TFs for which genes were observed in different times. Our results identified that network revealed the most significant differences among different genes.

Conclusion: Our findings would be useful for finding best time for treatment. We hope our study will be useful for generation of adiposity cells from MSCs.

Keywords: Rosiglitazone, Mesenchymal Stem Cells, Gephi Software

Ps-20: Comparison of Multipotency and Pluripotency Potential in Zebrafish Mesenchymal Stem Cells from Heart and Liver Tissue

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Background: There has been an explosion of research, publications and conference in the field of stem cells in the last decade. Mesenchymal stem cells (MSCs) are undifferentiated cells found in many adult tissues, including adipose, bone marrow, brain, muscle, ocular, heart, liver etc. In addition, MSCs have been considered as an appropriate source for gene and cell therapy tools for tissue engineering and regenerative medicine. To clarify different aspects of MSCs biology and develop MSCs applications in human diseases treatment, other studies on animal models are required. However, the similarity between zebrafish and human genome has been further examined; among other animal models that have been used, zebrafish is considered to be the best for practical purposes in regenerative medicine. In this report, multi-potency and pluri-potency potential of heart and liver derived-MSCs was presented for the first time. The aim of this study was to investigate the multipotency and pluripotency of zebrafish heart and liver derived-MSCs for using zebrafish as suitable animal model for regenerative medicine in cell therapy.

Materials and Methods: In the present study, MSCs were isolated from heart and liver tissue of Zebrafish (*Danio rerio*) as previously reported in another papers. The flow-cytometry as well as RT-PCR was used to analyze the expression of a panel of cell surface markers CD44, CD90, CD31 and CD34. In the following, alizarin red, oil Red-O and toluidine blue staining were carried out to evaluate the multipotency of zebrafish heart and liver tissue-derived MSCs. Subsequently, the gene expressions of Oct4, Sox2 and Nanog as pluri-potent markers were analyzed by RT-PCR.

Results: The results showed that, like other MSCs, zebrafish heart and liver tissue-derived MSCs were expressed pluripotent markers Oct4, Sox2 and Nanog. Moreover, these cells were differentiated to osteocyte, adipocyte and chondrocyte lineages following directed differentiation. However, the findings of this study are consistent with the hypothesis that MSCs have similar features with other MSCs in different tissue.

Conclusion: Our results show that both heart and liver tissue have similar characteristics including multi-lineage differentiation capacity and pluripotency potential.

Keywords: Zebrafish, Mesenchymal Stem Cells, Pluripotency, Multipotency

Ps-21: Stimulatory Effect of Bone Marrow Mesenchymal Stem Cell Co-Culture on Apoptosis and Cell Cycle Arrest of K562 Cell Line

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Background: Bone marrow derived mesenchymal stem cells (BMSCs) as one of the most common MSCs, reside in the bone marrow and control the homing and differentiation process of hematopoiesis. They are an excellent instrument for cell ther-

apy, regenerative medicine and co-culture with hematopoietic stem cells (HSCs). BMSCs derived from adults produce signals for proliferation and differentiation of HSCs and their progenitors during direct cell-cell contact. MSCs are injected parallel to HSCs to enhance bone marrow transplantations. However, the effects of MSCs on hematopoietic cell differentiation and possible precise cellular pathways are yet to well understood. Therefore, in this study we have reported the effect of MSCs on cell cycling and apoptosis through Annexin V binding of co-cultured K562 cell line.

Materials and Methods: In this experimental study, BMSCs were extracted from bone marrow of *rattus norvegicus*, subsequently, K562 as chronic myeloid leukemia cell line were purchased from Pasteur Institute and cultured till sub-confluent condition. At day 7, cultured K562 cell line alone and co-cultured K562 and with BMSCs (10:1) were collected and the cell number was counted. Finally, the average value of the cell number was calculated and growth curves were plotted. In the following, K562 cells were incubated 100 μ l binding buffer containing 5 μ l of FITC-conjugated Annexin V for 15 min at 25°C. Next, cells were washed with binding buffer and exposed with 5 μ l of propidium iodide (PI) solution in 100 μ l binding buffer. Flow cytometry was performed by FACSCalibur. Also, for cell cycle analysis, K562 cells were washed in cold PBS, fixed by ice-cold ethanol (70% w/w) and then incubated in PBS containing 0.1%, Triton X-100, 0.1% sodium citrate, and PI (50 μ g/ml; Sigma) at 4 °C for 30 min. Finally, kinetic of cell cycle was analyzed by flow cytometry method using a BD FACSCalibur system.

Results: The results of the present study showed that BMSCs have potent capability to induce early and late apoptotic as well as necrotic changes through 7 days' incubation. Also, the predominant effect of BMSCs on inhibition of cell proliferation was also detected by Ki-67 expression. The cell cycling kinetics analysis of K562 cells being exposed to BMSCs also prominent increase in percentage of G0/G1 phase cells.

Conclusion: Taken together, the data indicated that BMSCs significantly suppressed erythropoiesis via stimulation of apoptosis and cell cycle arrest of K562.

Keywords: Bone Marrow Mesenchymal Stem Cell, Co-Culture, Cell Cycling Kinetics, Apoptosis

Ps-22: Influence of Cytokines Secreted by Bone Marrow Mesenchymal Stem Cells on Cell Proliferation of K562 Cell Line

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Background: Bone marrow derived-mesenchymal stem cells (BMSCs), as kind of stem cells differing from hematopoietic stem cells, are weakly distributed within bone marrow with broad view for the therapeutic applications such as hematopoiesis regulation and differentiation, tissue engineering, regenerative medicine and cell transplantation. Previous studies have found that attached co-culture of cancer cell lines with normal adult bone marrow mesenchymal cells leads to an increase of

cells in G1 phase, a decrease of cells in S phase and directly inhibit the proliferation of leukemia cells and block cell cycle. But the cellular pathway details have yet to be reported. It was indicated that MSCs could mediate the suppression of cancer cell through secreting soluble cytokines. Therefore, MSCs attract great interest of many researchers in the fields of cell transplantation and cancer therapy. In the present study we have examined the effect of cytokines secreted by MSCs on cell proliferation of co-cultured K562 cell line.

Materials and Methods: After isolation of BMSCs from the bone marrow, these cells were characterized by flow cytometry using a panel of monoclonal antibodies and were tested for their potential to differentiate along different mesenchymal lineages. In the following, cryopreserved K562 cell line was routinely recovered and the number of live cells was counted using Trypan blue staining. BMSCs of the third passage was trypsinized using trypsin-EDTA, collected and plated into 6-well co-culture plate at 10×10^4 cells/per well. After 24 hours, 1 mL cell suspension of K562 at a density of 10×10^5 cells/per well was added into co-culture plate. At the end of seventh day, cultured K562 cell line alone and co-cultured K562 and with BMSCs (10:1) were collected, the cell number was counted and cell proliferation was assessed by flow cytometry. Subsequently, supernatant exposed in both groups (cultured K562 cell line alone and co-cultured K562 and with BMSCs) was collected and cytokine array antibody array was done.

Results: The results of this study showed that passaged BMSCs were uniformly fibroblast-like cells with a latent period of 19–24h. The positive rate of CD44 was 97.6% and that of CD45 and CD34 was negative on the cell surfaces of the third passage of BMSCs. In addition, antibody cytokine array showed that some cytokines such as IL-6 and IL-10 was highly expressed in co-cultured BMSCs and K562 cell line supernatant as compared with control group.

Conclusion: In this experiment, it was examined that BMSCs could suppress cell proliferation of K562 cell line via secreted cytokines including, IL-6, IL-10 and so on.

Keywords: Antibody Cytokine Array, Cell Proliferation, Bone Marrow Mesenchymal Stem Cell, K562 Cell Line

Ps-23: Examining Nuclear Membrane Integrity upon Cistaosis

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Background: It has been shown that Tau phosphorylation, a phosphoprotein belonging to the Microtubule Associated Proteins family, on threonine 231 causes the position of Cis or Trans conformations and while trans play a physiological role, Cis is highly toxic and the transfer of Cis p-Tau-231 to the nucleus leads to cell death. here is evidence about the role of nuclear membrane integrity in the death of neurons before and after the Cis p-Tau translocation to the nucleus.

Materials and Methods: This led us to examine the integrity of the membrane during cistaosis. On the other hand, we plan to examine the effect of nuclear membrane stabilization on reducing the death of neurons in *in vivo* and *in vitro* conditions.

Results: We showed that the nuclear membrane in SH-SY5Y and mice primary neuron during Cistaosis is naïve and the

membrane destruction accrue after a while after Cis P-tau231 translocation to the nucleus. Furthermore, treatment with nucleus membrane stabilizers including Green Tea Extract, Curcumin and Valproate Sodium can reduce cell death *in vitro* and *in vivo* and it has been seen improvements in behavioral assessments on C57/BL61 model of TBI.

Conclusion: Given that cispT231 tau has been evaluated as a major neurotoxic factor for Tauopathy disorders, it was critical to explore its neurotoxic effects using comprehensive procedures. Also it seemed reasonable to address whether it plays the neurotoxic roles after nuclear localization to find an efficient therapeutic strategy. These chemicals can employ in future studies in order to reduce side effects of Tauopathies such as TBI, Alzheimer and so on.

Keywords: CispT231, Cistaosis, Laminopathy, Nuclear Membrane, TBI

Ps-24: Epigenome and Transcriptome Analysis Revealed Core Regulatory Network Involved in Glioblastoma

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Background: Glioblastoma is a main type of brain tumors that derived from astrocytes. Glioblastoma are classified as very aggressive cancers with no clear cause. Transcriptome of the cancerous cells dramatically differs from normal ones. On the other hand, role of epigenetic alterations has been shown in cancer development. Here, using bioinformatics and biostatistics, we have analyzed transcriptome and epigenome data of 4 glioblastoma specimens to reveal main transcription factors (TFs) and their epigenetic status. Additionally, we have constructed a core regulatory network for glioblastoma.

Materials and Methods: To do this study, we used dataset GSE46016 which covers several samples for transcriptome, methylome, and histone modification data for 4 brain tumor samples, neural stem cells and normal astrocytes samples. Using GEO2R tool of NCBI compared tumor samples against astrocytes and applying FDR equal or less than 0.01 and log₂FC 0.6 and 1.5 DE genes were detected. We used hierarchical clustering to cluster the TFs and predict differentially expressed TFs (DE-TFs), which are controlling all other TFs using Enrichr database. Core regulatory network of main TFs were constructed and analyzed with Cytoscape 3.4.0 program to find hub TFs. Afterward, methylation status and histone modifications were considered to confirm transcriptome findings.

Results: Our analysis revealed that there are 119 common TFs in all tumor samples. Using data of ChEA and ENCODE databases, we predicted 22 DE-TFs controlling all other TFs. Despite of almost revers pattern of gene expression in one of the samples, we found 7 TFs, including ZNF217, TCF4, and JUN, up-regulated in glioblastoma. Almost all target genes of these TFs were up-regulated as well. Gene ontology revealed that this genes are related to apoptosis and neuron differentiation and migration. Methylome analysis of upstream these TFs showed hypomethylation of DMRs. H3K27me₃ and H3K4me₃ modifications were majors for most of the TFs.

Conclusion: Using transcriptome and epigenome data, we have detected several hub TFs significantly involved in glioblasto-

ma, which confirm previous findings and also predict potentially important candidates for further studies.

Keywords: Bioinformatics, Epigenome, Glioblastoma, Stem Cell, Transcriptome

Ps-25: Signed weighted Gene Co-Expression Network Analysis Revealed Systems Level Characteristics of Parkinson's Disease

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Background: Parkinson's disease as a progressive neurodegenerative disease involves central nervous system with consequent progressive neurological symptoms including behavioral and cognitive disabilities. This disease causes many yearly new cases worldwide and result in mortality in some years after symptoms appearance. Here, we used weighted gene co-expression network analysis (WGCNA) to detect highly important (hub) genes involved in the disease.

Materials and Methods: We used GSE68719 dataset contains 29 disease and 44 healthy control post-mortem samples from prefrontal cortex of human brain. Matrix was refined for proper genes using removing duplicate genes and analyzing coefficient of variation. To analyze WGCNA, we used R programming package with the same name. DAVID database was used to analyze gene ontology. Clustering analysis were done by heatmap.3 package of R program. Cytoscape 3.4.0 and Gephi 0.9 programs were used to final analyze and visualize the networks.

Results: Our results showed 6 distinct modules that were highly related to the disease. Gene ontology revealed that these modules are related to chemical synaptic transmission, regulation of transcription, DNA-templated, and inflammatory response. Clustering of hub genes almost divided controls and disease samples into distinct groups. Centrality analysis of the modules detected several genes including RPL13, TPRA1, SLC25A39, and DMPK as hub genes.

Conclusion: Our study revealed Parkinson's disease characteristics in the systems level and several hub genes which could be used for further studies as markers or therapeutic targets.

Keywords: Clustering, Gene Ontology, Hub Genes, Parkinson's Disease, WGCNA

Ps-26: Encapsulation of Rat Pancreatic Islets by Sulfated Alginate in Order to Reduce The Fibrosis Against Transplanted Microcapsules

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Background: Encapsulation of islets has been proposed as a potential treatment for type 1 diabetes disease to overcome the immune-mediated destruction of the graft without the need for anti-rejection drugs. From a wide range of different polymers,

alginate is introduced as an ideal biomaterial for the encapsulation of pancreatic islets. Nevertheless, the fibrotic tissue overgrowth on alginate capsules still remains as one of the challenges in the successful application of this promising technology. In this study, we used sulfated alginate to inhibit or reduce the creation of fibrotic responses against transplanted islets.

Materials and Methods: We used two newly synthesized sulfated alginate polymers. To determine the presence of the sulfate groups in alginate backbone, FTIR test (Fourier-transform infrared spectroscopy) has been done. A-PLL-SA (Alginate-Poly l lysine -Sulfated Alginate) microcapsules were generated by peristaltic pump and FITC labeling (Fluorescein isothiocyanate) was used to demonstrate PLL (Poly l lysine) and SA (Sulfated Alginate) presence on microcapsules. Islets have been encapsulated in A-PLL-SA (Alginate-Poly l lysine -Sulfated Alginate) capsules and fluorescein diacetate (FDA) and propidium iodide (PI) staining applied to evaluate the effect of sulfate groups on encapsulated islets viability. GSIS test (Glucose-stimulated insulin secretion) was used to evaluate the function of islets after encapsulation. Encapsulated islets were transplanted in diabetic rats and we retrieved them after 28 days. Then the level of collagen around retrieved microcapsules was identified by MT staining (Masson's trichrome).

Results: The FTIR test (Fourier-transform infrared spectroscopy) of sulfated alginate determined the presence of sulfate groups by showing absorption spectra in 1260 wavelength. Immunofluorescence imaging of FITC (Fluorescein isothiocyanate) labeled capsules confirmed the presence of PLL and SA layer. Fluorescein diacetate (FDA) and propidium iodide (PI) staining and GSIS (Glucose-stimulated insulin secretion) test did not show the negative effects of sulfated alginate on viability and functionality of encapsulated islets. After graft retrieval, MT (Masson's trichrome) staining demonstrated the reduction of collagen levels around transplanted microcapsules.

Conclusion: This study showed that using a modified material could reduce the fibrosis against transplanted islet, paving the road to an efficient islet transplantation procedure.

Keywords: Encapsulated Islet, Fibrosis Reduction, Islet Transplantation, Alginate Modification

Ps-27: MiR- 202: A New Regulator of Myblast to Myocyte Differentiation Process

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Background: Myogenesis is a specific multi-step differentiation process which is performed in embryonic and postnatal stages. Recently, it has been reported that Rho-associated kinase 1 (ROCK-1), have a negative regulatory function in myogenic differentiation. Complicated controlling mechanisms of myogenesis process are applied by various factors. Lately, investigators are interested in discovering microRNAs (small non-coding RNAs that play crucial roles in numerous biological processes) and their relationship in myogenesis. In this survey, we aimed to explore the impact of microRNA-202 as a bioinformatic regulator of Rock in myogenesis.

Materials and Methods: This experimental study was con-

ducted on the C2C12 cell line. Using a bioinformatics attitude, miR-202 was selected based on its target as potential factor in myoblast to myocyte differentiation induced by 3% horse serum. Immunocytochemistry (ICC) was carried out to confirm the differentiation process and quantitative real-time polymerase chain reaction (PCR) to evaluate the expression level of miRNA and its target. The Dual luciferase assay was performed to confirm the direct effect of miR-202 on Rock-1 expression.

Results: During myoblast to myocyte differentiation, miR-202 was significantly up regulated while Rock1 was down regulated during the process. Subsequently, using dual luciferase reporter assay analysis Rock-1 was validated as miR-202-1 target.

Conclusion: To sum up miR-202 is a potential regulator of myogenesis and is involved in skeletal muscle development through suppressing the Rock-1 pathway.

Keywords: miR-202, Myoblast, Differentiation, Rock-1

Ps-28: *In Vitro* Evaluation of A Rapidly In Situ Forming Hyaluronic Acid Hydrogel as A Carrier for Mesenchymal Stem Cell Delivery

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Background: In situ delivery of cells is expected to enhance tissue regeneration, especially delivery of mesenchymal stem cells (MSC) because they can differentiate to many cell types. To have a proper therapeutic effects, cells should remain alive and metabolically active in the defect site, thus having a good carrier for cell delivery is crucial. In this study, an injectable hyaluronic acid-tyramine (HA-TA) hydrogel was developed for delivery of human MSCs, which is applied in a target area with a one-step minimal invasive method.

Materials and Methods: The HA-TA conjugate was synthesized based on EDC/NHS chemistry. The HA-TA was enzymatically cross-linked via an oxidation reaction by addition of Horseradish peroxidase (HRP) and hydrogen peroxide (H₂O₂) in 5 to 15 seconds. The hMSCs was isolated from bone marrow and were incorporated in the hydrogel during the gelation procedure. To enhance the bioactivity of the hydrogel, platelet lysate (PL) was incorporated into the constructs. Platelet lysate is a kind of hemoderivatives which is obtained by repeated freezing and thawing of the platelet concentrates, and is an autologous and inexpensive source of abundant growth factors and cytokines. In spite of good biocompatibility of the HA-TA hydrogel, it lacks cell binding sites. Unlike that, PL derived hydrogels are rich in growth factors and biological signals but does not have mechanical stability. The hypothesis is to combine the advantages of these two systems and propose a hydrogel which can be easily used in clinical setting.

Results: The HA-TA was synthesized with the degree of substitution of 13 TA moieties per 100 disaccharide units. The storage moduli of the HA-TA hydrogels ranged from 500 to 2000 Pa and increased with increasing polymer concentration. Encapsulating the cells in injectable HA-TA hydrogel showed that the hMSCs remained viable for at least one month, but they could

not proliferate. The mild gelation condition helps the cells to remain alive during crosslinking procedure. In order to provide the biological signals and cell binding sites in the hydrogel, platelet lysate was successfully incorporated in the hydrogels. Addition of the PL did not change the physical properties of the hydrogels. In contrast to a retained round shape of the cells in HA-TA hydrogel, the hMSCs spread in the PL enriched hydrogels, and also they proliferated. The metabolic activity of the cells in constructs containing PL was much higher compared to the pure HA-TA hydrogels.

Conclusion: In this study, an enzymatically crosslinked HA-TA hydrogel was complemented with platelet lysate as an autologous source of growth factors and cytokines. Based on the achieved data, this injectable hydrogel is an easy-to-apply approach and shows high potential as a cell delivery carrier for tissue regeneration.

Keywords: Enzymatic Crosslinking, Platelet Lysate, Injectable Hydrogel, Hyaluronic Acid, Mesenchymal Stem Cell

Ps-29: Neural Stem Cells at Sleep: Selective inhibition of Orexin 2 Receptor in Mouse Cortical Neural Stem Cells Increases Proliferation and Differentiation toward Oligodendrocytes

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Background: In multiple sclerosis Oligodendrocytes are obliterated by the immune system. neural stem/ progenitor cells have the capacity to differentiate into mature myelinating oligodendrocytes. In embryonic mouse cortex oligodendrocyte progenitor cells are more abundant than the ganglionic eminence. Doing gene set enrichment analysis using DAVID and Panther websites it was shown that Gpr3711 is highly expressed in oligodendrocyte progenitor cells in comparison to oligodendrocytes (more than 300 fold change in expression). The selective orexin 2 receptor (HCRTR2) antagonist jnj-10397049 has been shown to inhibit this orphan GPCR[4]. In this study we sought to scrutinize embryonic cortical neural stem cells survival and differentiation behavior after inhibition of gpr3711 and HCRTR2 by jnj-10397049.

Materials and Methods: Gene set enrichment and pathway analysis was done using Panther and David websites. Primary cortex neural stem cells were derived from embryonic mouse 13.5 and cultured in complete neural stem cell proliferation medium. All assays were done after passage 2. cytotoxicity effect of varying doses of JNJ10397049 was screened using MTT assay. The expression of gpr3711, hcrr2, PDGFRalpha and Cnpase expression was analyzed using real time PCR in cortical and ganglionic eminence neural stem cells. For differentiation analysis GFAP, Cnpase and PDGFRalpha expression was analyzed using Real-time PCR on day 3 and 7 after treatment

with 10, 15 and 20 micro-molar of JNJ10397049. Expression of Olig2, beta III tubulin, Myelin Basic Protein and nestin on day 7 after differentiation induction of shown by Immunocytochemistry.

Results: MTT analysis demonstrated that JNJ10397049 at 15 and 10 micromolar dramatically increases proliferation of neural stem cells by 2.62 and 2.43 respectively. Gpr37l1 and orexin2 receptor are more expressed in embryonic mouse cortex NS/PCs than embryonic mouse ganglionic eminence by 3.45 and 4.57 ,respectively. PDGFRalpha and Cnase genes are also highly expressed on cortex NS/PCs by 112.36 and 76.56, respectively in comparison to ganglionic eminence NS/PCs. The inhibitor after 7 days at dose 15 micromolar increased the expression of Cnase, PDGFRalpha and GFAP by 1 , 20.56 and 1.47 fold, respectively. The expression of Olig2 was prominent at 15 micromolar, yet neither beta III tubulin nor nestin presence was observed at this dose.

Conclusion: In our experiment it was outlined that selective inhibition of orexin 2 receptor prominently increased not only the proliferation of Cortical neural stem cells which are enriched with oligodendrocyte progenitor cells but also enhances the differentiation of these cells toward Oligodendrocytes. Inhibition of Orexin 2 receptor can be a valid drug target in inducing remyelination. In an ongoing research by our department by CRISPR-mediated Knock-out of GPR37L1 we're sought to test whether JNJ10397049 exerts its effect only through inhibition of Orexin 2 receptor or also by inhibiting GPR37L1 as well.

Keywords: Cortical Neural Stem Cells, Oligodendrocytes, GPR37L1, Orexin Receptor, Multiple Scler

Ps-30: RISPR-Mediated Knock out of GPR37L1 in Oligodendrocyte Progenitor Cells: A Promising Therapeutic Target for Inducing Remyelination

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Background: In demyelinating diseases such as multiple sclerosis Oligodendrocytes are lost. An up-and-coming restorative strategy is inducing remyelination. Oligodendrocyte Progenitor Cells differentiation to oligodendrocytes (myelin-producing cells) is impeded by various factors. Doing gene set enrichment analysis using DAVID and Panther websites it was shown that Gpr37l1 is highly expressed in oligodendrocyte progenitor cells in comparison to oligodendrocytes (more than 300 fold change in expression). The selective orexin 2 receptor (HCRTR2) antagonist jnj-10397049 has been shown to inhibit this orphan GPCR. In a previous unpublished study it was shown that inhibition of HCRTR2 would increase Proliferation and Differentiation of cortical neural stem cells toward oligodendrocytes. To further analyzed the effect of this inhibitor and also to investigate the potential of GPR3L1 is a constitutively expressed receptor) in induction of oligodendrogenesis we intended on

knocking out this gene using CRISPR/Cas9 in oligodendrocyte progenitor cells.

Materials and Methods: Gene set enrichment and pathway analysis was done using Panther and David websites. More than 40% of marketed drugs target G-protein coupled receptors. Top 500 G-protein Coupled receptor Genes expressed in Oligodendrocyte progenitor cells in comparison to oligodendrocytes was selected. Oligodendrocyte progenitor cells were isolated using FACS with pdgralpha monoclonal antibody and cultured with 20 ng/μl bFGF, 10 ng/μl PDGFAA, 1% B27 and DMEM/F12 medium. Guide rna was designed using CRISPRko. Vectors containing spcas9 and GPR37L1 guide RNA is going to be transduced to Oligodendrocyte Progenitor Cells using lentiviral vectors.

Results: 15 most enriched GPCRs in Oligodendrocyte progenitor cells was chosen out of the 500 expressed genes (Chrm1, Chrm2, Hrh1, Chrna4, Gpr37L1, Ednrb, P2ry1, Gpr17, Gpr37, Gpr162, Gpr 19, Gpr34, Gpr56, Gpr62). Gpr17 is highly expressed in newly formed oligodendrocytes with more than 900 fold change in expression in comparison to mature myelinating Oligodendrocytes. Endothelin b receptor and Gpr37 has already been shown to negatively regulated Oligodendrocyte differentiation. GPR37L1 has more than 300 fold change in expression in oligodendrocyte progenitor cells in comparison to oligodendrocytes. Further analysis hasn't been done yet.

Conclusion: GPR37L1 is a promising target in inducing oligodendrocyte differentiation. Further analysis to confirm this hypothesis using CRISPR-mediated knockout and the suggested inhibitor of this receptor (JNJ10397049) is underway. Also to validate the suggested orexin 2 receptor inhibitor as a ligand for GPR37L1 (a constitutive orphan GPCR) in an article published in 2017, and to inspect our previous result showing increased proliferation and oligodendrocyte differentiation of cortical neural stem cells the current ongoing experiment is being carried out.

Keywords: CRISPR/Cas9, Oligodendrocytes, GPR37L1, Remyelination

Ps-31: In Vitro and In Vivo Proliferative Activity of Royan DiaHerb 1092 on Pancreatic Beta Cells

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Background: Type 1 diabetes mellitus (T1DM) and the late stages of Type 2 diabetes mellitus (T2DM) are resulted from inadequate insulin action, a consequence of loss of functional beta-cells. The loss of beta cell mass combined with beta-cell dysfunction is caused by autoimmune destruction in T1DM, while by metabolic exhaustion in T2DM. Preservation and expansion of pancreatic beta cell mass could be an effective therapeutic approach. However, this approach is challenging since the low capacity of beta-cell regeneration caused to be considered as a quiescent cell type. The global use of complementary and alternative medicine (CAM) for the management of diseases such as diabetes has rapidly increased over the last

decade. It is reported that up to 72.8% of people with diabetes used herbal medicine and dietary supplements. The ethnobotanical information suggests that about 800 plants may possess anti-diabetic potential.

Materials and Methods: In this study, we developed an *in vitro* and *vivo* models to study regenerative effects of a herbal extract named RoyanDiaHerb 1092 (RDH) on pancreatic islet cells. For extraction, the RDH plant was collected from the research field of Faculty of Agriculture, Tarbiat Modares University and then dried in shadow and 10 g of crushed material was sonicated for 30 min in 50:50 water and ethanol to obtain extracts. To develop the *in vitro* model of beta cell proliferation, islets were isolated from rat pancreas tissue using our previously published protocol. Islet cells were dissociated by trypsin and cultured with a defined cell density in 96-well plates. Cells were cultured overnight in Ham's F10 supplemented by fetal bovine serum at 37°C and 5% CO₂. The RDH extract was then added to islet-derived cells' media at different concentrations (30, 100, 1000 and 10000 mg/ml) for 6 days. To investigate the proliferative effects, treated cells were immuno-stained by anti-insulin and anti Ki67 antibodies and were imaged. The percentage of both insulin+ and Ki67+ (insulin+/Ki67+) cells were reported as the proliferative efficiency of the RDH. For *in vivo* model, we used Tg(Pdx1:EGFP) mice (produced by Royan Gene Targeting Laboratory). After intra-peritoneal injection of the extract for 15 days mice were sacrificed at day 30 of the experiment and the beta cell mass of the pancreas were measured by processing of the fluorescent microscopy images taken from the whole pancreas.

Results: Our results from the *in vitro* model showed significant increase in the insulin+/Ki67+ cells in the group treated with 30 to 1000 µg/ml RDH extract comparing to the negative control group. Our preliminary *in vivo* data showed a significant decrease in fasting blood glucose levels of the Tg mice treated with RDH.

Conclusion: While further characterization and more *in vivo* experiments needed to confirm the regenerative effects of RDH extract in beta-cells, our data reflect a promising therapeutic ability for RDH to treat T1DM.

Keywords: Beta Cell, Proliferation, Herbal Extract

Ps-32: Anti-Cancer Effects of MicroRNA-200c in Colorectal Cancer via Cancer Stem Cell Marker B-Cell-Specific Moloney Murine Leukemia Virus Integration Site 1

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Background: Colorectal cancer (CRC) is one of the most leading cancer deaths throughout the world. Surgery, chemotherapy and radiotherapy are the current treatment for CRC. Despite advances in diagnosis and treatment, the mortality rate is high. Therefore, finding appropriate biomarkers for timely detection and treatment is necessary. CRC stem cell share many similar characteristics of normal intestinal stem cells and are hypothesized originate from them. BMI1 surfaces as a bio-signature of CSCs. BMI1 is a member of polycomb repressive complex that has multiple role. As a new paradigm for therapy resistance, the role of BMI1 in this perspective is also highlighted. miRNAs are small endogenous noncoding RNAs that regulated gene expression. In this study, we investigated the miR-200c

expression in CRC tissues and its effects in CRC cell lines that mediated by BMI1.

Materials and Methods: QRT-PCR and immunohistochemistry were employed to detect miR-200c and BMI1 expression in tumor tissues from 38 patients with CRC and 38 non-cancerous tissues. HCT-116 and SW-48 cells were transfected by LNA-anti-miR-200c. Western blot and real-time PCR were applied to determine the BMI1 protein and miRNA levels. The apoptosis was analyzed via Annexin/ PI staining and cell invasion was evaluated by Transwell assay.

Results: miR-200c was markedly down regulated in CRC tissues whereas protein expression of BMI1 in CRC tissues was up regulated compared with non-cancerous tissues. In the colon cancer cell lines, transfection of LNA-anti-miR-200c increased BMI1 gene and protein expression as well as the cell invasion. Down regulation of miR-200c by LNA decreased the apoptotic cells.

Conclusion: Our results showed the down regulation of miR-200c increases the invasion of tumor cells and decrease the rate of apoptosis. Moreover, the expression of miR-200c has a reverse relationship with BMI1. Finally, our finding indicates that miR-200c acts as tumor suppressor in CRC through inhibiting of BMI1 expression.

Keywords: Colorectal Cancer, Cancer Stem Cell, BMI1, miR-200c, Apoptosis

Ps-33: Over Expression of Leukemia Inhibitory Factor Upregulates the Expression of Adhesion Molecules and Cytokine Receptors in Adipocyte Derived Mesenchymal Stem Cells

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Background: The use of adipose tissue derived mesenchymal stem cells (ADMSCs) for the treatment of central nervous system diseases and anti cancer drug delivery in animal and clinical human researches has been attention during the recent years. The limitation of stem cell migration to the brain is one of the most important barriers to effective treatment with stem cells. Therefore, many studies have been done to understand the mechanisms and functions of genes which related to stem cell migration into the brain. In previous studies, the effect of leukemia inhibitor factor (LIF) on the potency of stem cells, various stages of neurogenesis and repair of brain and spinal cord injury have been shown. The purpose of the present study was to evaluate the effect of over expression of LIF in ADMSCs cells on the gene expression of adhesion molecules and cytokine receptors relate to the transmission of stem cells from the blood-brain barrier.

Materials and Methods: ADMSCs was expanded from pretesticular adipose tissue of male C57BL/6 mice based on digestion with collagenase I and serial passage in DMEM medium containing 15% FCS and antibiotics. Isolated cells were phe-

notypically characterized based on determination of expression of OCT4 and NANOG genes by conventional PCR, presence or absence of some surface markers including CD90, CD44, CD31 & CD45. In the present study ADMSCs was transfected by Eukaryotic pIRES2-EGFP vector containing LIF gene using calcium-phosphate method. Expression of CXCR-1, CXCR-4, CCR-2, LFA-1, MAC-1, VLA-4 & PSGL-1 and was analyzed using real time PCR at days 7 and 14 after transfection. Non transfected ADMSCs were used as negative control.

Results: Our results showed that CXCR-1, CXCR-4, CCR-2, LFA-1, MAC-1, VLA-4 were transcribed in ADMSCs, while PSGL1 had no expression in ADMSCs. Over expression of LIF in ADMSCs resulted in elevation of expression of all studied adhesion molecules and cytokine receptors, in particular, 14 days after transfection.

Conclusion: Given the stimulatory effects of LIF overexpression on the transcription of adhesion molecules and cytokine receptors genes in ADMSCs, It is likely that this method can be used to improve the transmission of stem cells for treatment of neurodegenerative diseases in future.

Keywords: Adipocyte Derived Mesenchymal Stem Cells, Adhesion Molecules, Cytokine Receptors, Leukemia Inhibitory Factor

Ps-34: The Novel Approach to Design A Bioengineered Scaffold for Corneal Regeneration

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Background: Using 3D bioprinting in tissue engineering is one of the newest approaches to regenerate the injured tissue. Natural biomaterials are known to be a perfect bioink for 3D bioprinting, since they are cyto-compatible and show less inflammatory response. The purpose of this study is to design a proper scaffold for corneal regeneration using gelatin methacrylate (GelMA) in 3D bioprinting technique. In this case, the geometry of the human cornea is designed and sliced by proper software and reflected to the bioink with visible light.

Materials and Methods: Gelatin type A from porcine skin, Glycidyl methacrylate, Eosin Y disodium salt (Eosin Y), 4-(dimethylamino) pyridine (DMAP), and dimethyl sulfoxide (DMSO) are purchased from Sigma Aldrich. GelMA is prepared as follows: 5 g gelatin is dissolved in 50 ml DMSO with stirring at 50°C. Then, DMAP is added to the mixture and dissolved. Subsequently, glycidyl methacrylate is added and the mixture is stirred for two days at 50°C. The mixture is then dialyzed with reverse osmosis (RO) water at room temperature for five days. The water is changed every day and after dialysis, a freeze-dried sample is achieved via lyophilization. For preparing photocrosslinkable bioink, freeze-dried GelMA is dissolved with Eosin Y in PBS and stem cells are added to the solution. Subsequently, the geometry of the cornea is printed layer by layer exposing the bioink to visible light. The crosslinking time, the bioink volume and thickness of each layer is set to develop the normal round shape of the cornea.

Results: The geometry of the cornea is printed at the same size of the human cornea. Although the hydrogel is printed layer by layer, more than 90% optical transparency is achieved. Moreover, 94% and 93% cell viability is observed for day 0 and day 7 respectively. Furthermore, a complete cell alignment is ob-

tained in each layer after a week.

Conclusion: The results showed that GelMA is a proper bioink for 3D bioprinting the cornea geometry and the layer by layer bioprinting didn't have negative effect on cell viability and proliferation.

Keywords: 3D Bioprinting, Cornea Regeneration, Biomaterial, Gelatin Methacrylate

Ps-35: Targeting The Core Regulatory Network of Stemness in An *In Vitro* AML Model

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Background: Acute myelogenous leukemia (AML) is considered as one of the most common types of hemato-oncology deficiencies in adults. Despite the use of aggressive chemotherapy, relapse after remission and refractoriness to induction chemotherapy still remain as the most common therapeutic failures in AML. It seems that a rare population of cells called Leukemia stem cells (LSCs), are responsible for initiating therapy resistance and relapse. Gene Regulatory Network (GRN) is an adequate bioinformatics tool used in mapping gene interactions and also, pinpointing key genes which may regulate the stemness core in LSC. Therefore, in this study we applied GRN to identify a precise list of potential biomarkers and target genes, which may play an effective role in targeting these cells.

Materials and Methods: This study was conducted in two phases, 1) *in silico* 2) *in vitro*. In the *in silico* phase our aim was to identify key genes and their related signaling pathways which may be regulating the stemness core in LSCs. In this phase, we obtained a data set of 495 Gene expression profiles (GSE76009). Differentially expression analysis was performed between two defined groups of LSC+ and LSC- using limma package in R Software (3.4.2 version). In addition, network construction was conducted using ARACNE algorithm and cytoscape software. Subsequently, 24 "Hub genes", genes which have the most interaction with other genes, were identified and pathway analysis was conducted using different tools in different databases. Hence, based on our selection criteria, the list of genes was narrowed down to 4 genes, to be evaluated in the wet-lab phase. In the *in vitro* phase, two approaches were followed. First, we used two small molecules SANT-1 and RG108 to inhibit two important signaling pathways in KG-1 cell line. In the second step, KG-1 cell line was transfected with a combination of four siRNAs to evaluate stemness decrease. In the *in silico* phase we determined 4 genes and their related signaling pathways. Moreover, we indicated that by using the combination of SANT-1 and RG108, a significant decrease in stemness is observed. We also observed that, the combinational inhibition of 3 siRNAs had a significant role in decreasing stemness. By comparing the two inhibitory methods, siRNA transfection exhibited more effective results.

Results: We determined a candidate set of genes that are significantly related to stemness and self renewal pathways in AML. **Conclusion:** Our data suggested that by using this bioinformatics approach, we were able to attain a list of candidate genes which may have a significant role in regulating the stemness core in LSCs.

Keywords: AML, Gene Regulatory Network, Leukemia Stem Cells, Bioinformatics

Ps-36: Evaluation of Pulmonary Vascular Permeability following Using Intratracheal of Marrow-Derived Mesenchymal Stem Cells in Experimental Model of Acute Respiratory Distress Syndrome in Sheep

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Background: The acute respiratory distress syndrome (ARDS), is a clinical complication of severe acute lung Injury. ARDS is characterized by disruption of the lung alveolar-capillary membrane barrier and resultant pulmonary edema associated with a proteinaceous alveolar exudate. Current specific treatment strategies for ARDS are lacking. The use of mesenchymal stem cells in the treatment of lung injury and recovery has become a promising therapeutic approach. The purpose of this study is to evaluate the performance of bone marrow-derived mesenchymal stem cells (BM-MSCs) in pulmonary vascular permeability in ARDS model.

Materials and Methods: In this study, 10 male sheep 3-4 months old Shall strain were selected and were randomly divided into two groups of treatment and control. By intratracheal administration of E.Coli-lipopolysaccharide strain of O55:B5 has induced an experimental model of ARDS and confirmed by registration of clinical signs and radiographic image. Then in the treatment group, BM-MSCs were collected, isolated and cultivated. The number of cells for each sheep was brought to 5×10^7 cells. After 24 hours of ARDS-induce, BM-MSCs were autographed as intratracheal in the treatment group and in control-group 10 ml intratracheal PBS was injected. In order to evaluate effects of cell therapy, before and 24,48,72 and 168 hours after stem cell transplantation or PBS infusion samples of bronchoalveolar lavage(BAL)were obtained by bronchoscopy from distal airway to measure the concentration of IgM, albumin and total protein by ELISA Kit.

Results: The results showed that transplantation of stem cells could reduce the concentration of increased IgM, albumin and total protein in lung inflammation time, significantly. The ratio of IgM to total protein and albumin to total protein showed a significant reduction at 72 and 168 hours and total protein was decreased at hours of 48,72,168 compared with inflammation time($P < 0.05$).

Conclusion: These results suggested that BM-MSCs could im-

prove the capillary-alveolar barrier integrity and play a significant role in the repair of lung injury.

Keywords: ARDS, BM-MSCs, BAL, Sheep

Ps-37: Valproic Acid Upregulates SDF1 according to Mathematical Prediction

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Background: Acute kidney injury as a major health problem can be treatable through Identifying the molecular mechanisms of tissue regeneration. In a previous study, we measured the expression of SDF-1, a chemokine known to be up-regulated in injured tissues and playing an essential role in regeneration by recruiting stem cells. Unexpectedly, its declining trend was observed following kidney ischemia reperfusion (IR). To explain this observation, a mathematical model was constructed and the activation of histone deacetylase was proposed as the main reason for SDF-1 down-regulation. In order to experimentally verify this prediction, we have here assessed the effect of valproic acid (VPA), a potent histone deacetylase inhibitor, on the kinetics of SDF-1 expression and tissue repair parameters, in a mouse model of kidney IR.

Materials and Methods: Mice were subjected to IR or sham operation and received either a single dose of VPA or normal saline. The mRNA expression of Sdf-1 alpha, beta, and gamma were measured in a time-course manner. Also, serum urea, creatinine, and histopathology parameters were assessed.

Results: Considerable fluctuations were observed in sham groups that disappeared following IR. Where continuous declining trend shows the reproducibility of our previous data. VPA induced the over-expression of gamma, but not alpha and beta mRNA in IR mice. Although, not only did VPA not show any protective effect on kidney damage, it further aggravated the condition.

Conclusion: The overexpression of SDF-1 in response to VPA administration validates the mathematical model predictions indicating the potential of systems biology modeling tools for the prediction of biomedical phenomena. In addition, the time course follow-up of all experimental groups allowed for a realistic insight into the dynamics of the measured parameters and the discrimination between actual responses and intrinsic variations.

Keywords: Acute Kidney Injury, SDF-1, Histone Deacetylase, Valproic Acid

Ps-38: Cardiomyocyte Differentiation of Wharton'S Jelly Mesenchymal Stem Cells by Different Cocktaies: Based On 5-Azacytidine

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Background: Many studies have shown the ability of Wharton's Jelly Mesenchymal Stem Cells (WJMSCs) to differentiate into cardiomyocyte-like cells under various inductions. 5-azacytidine (5-aza) induces cardiomyocyte differentiation by activating extracellular regulated kinases. In the present studies we have investigated the effect of different cocktails of 5-aza for cardiomyocyte differentiation of WJMSCs.

Materials and Methods: WJMSCs were harvested by explant culture method and mesenchymal surface markers (CD34, CD44, CD45, CD73, CD90 and CD105) were characterized by flow cytometry of mesenchymal cell markers. Also, adipogenic and osteogenic differentiation was evaluated. For cardiomyocyte differentiation, 4 cocktails including: 1) [5-aza], 2) [5-aza + ascorbic acid (Asc)], 3) [5-aza + hypoxia (Hypo)], 4) [5-aza + Asc + Hypo] were examined. The expression of Connexin43, GATA-4 and troponin I (cTnI) was evaluated by real time PCR.

Results: The isolated spindle like cells did not express hematopoietic lineage markers such as CD34 but expressed CD44, CD45, CD73, CD90 and CD105. Also, the cells exhibited osteogenic and adipogenic differentiation ability. The expression of GATA-4 and cTnI was higher in [5-Aza + Asc] group, with GATA-4 as the significant cardiogenic marker. Connexin43 had higher expression in [5-Aza + Hypo] group without any significant difference.

Conclusion: GATA is one of the first implicated transcription factor in the regulation of cardiomyocyte differentiation. As GATA and cTnI had higher expression level in [Aza + Asc] group, we may conclude that this cocktail can affect cardiomyocyte differentiation. We also may conclude that 5-azacytidine along with ascorbic acid or hypoxia are more suitable inducers for cardiomyocyte differentiation than 5-azacytidine alone and (Aza + Asc) is better than (Aza + Hypo).

Keywords: Cardiomyocyte Differentiation, Wharton's Jelly Mesenchymal Stem Cells, 5-Azacytidine

Ps-39: Comparative Effect of Hypoxia and Ascorbic Acid for Cardiomyocyte Differentiation of Wharton'S Jelly Mesenchymal Stem Cells

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Background: 5-azacytidine is a major classical factor for myogenic induction, but this drug is toxic. Many studies investigate the efficacy of the other alternative factors for cardiomyocyte differentiation. The present study aims to survey the effect of various cocktails, containing hypoxia (Hypo) and ascorbic acid (Asc) for cardiomyocyte differentiation of Wharton's jelly mesenchymal stem cells (WJMSCs).

Materials and Methods: WJMSCs were isolated by explant culture. They were characterized by osteogenic and adipogenic differentiation and flow cytometry of mesenchymal markers. Heart conditioned medium (HCM) was collected from neonatal rat heart. 8 cocktail of 5-azacytidine (5-aza) and HCM were used including: 1) [HCM], 2) [HCM + Asc], 3) [HCM + Hypo], 4) [HCM + Asc + Hypo], 5) [5-aza], 6) [5-aza + Asc], 7) [5-aza + Hypo], 8) [5-aza + Asc + Hypo]. Then, the expression of α -actin and connexin43 was investigated by immunofluorescence. The equivalent groups were compared; (1 ~ 5), (2 ~ 6), (3 ~ 7) and (4 ~ 8).

Results: The cells were positive for mesenchymal markers (CD44, CD45, CD73, CD90 and CD105) and negative for CD34. They demonstrated adipogenic and osteogenic differentiation. In [HCM + Asc], α -actin had higher level than [5-Aza + Asc]. The comparison of [Hypo + Aza] and [Hypo + HCM] showed that cardiac markers had higher expression level in group containing 5-Aza, while the comparison of [5-aza + Asc + Hypo] and [HCM + Asc + Hypo] showed that connexin43 and α -actin had the most expression level in group containing HCM.

Conclusion: As, connexin43 and α -actin had the most expression level in group 4 and 6. It means that the cocktail of "Asc + Hypo" in combination with 5-Aza or HCM can be the best combination for cardiomyocyte differentiation. We can conclude that when hypoxia is combined with ascorbic acid, it can accomplish better cardiomyocyte differentiation than absence of ascorbic acid.

Keywords: Cardiomyocyte Differentiation, Wharton's Jelly Mesenchymal Stem Cells, Heart Conditioned Medium

Ps-40: Utilization of Laser Therapy on Bone Marrow-Derived Mesenchymal Stem Cells in An Experimental Osteoporotic Model

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Background: Osteoporosis is a common disorder characterized by systemic bone mass defect and its microstructure, resulting in bone fractures. Different research results show the positive effects of continuous lasers on accelerating the fracture healing in healthy animal models. Also, in other recent studies, low-power laser radiation increased the growth of rat and human like osteoblastic cells in the presence of dexamethasone.

MSCs are a proposed therapeutic tool in tissue rehabilitation and tissue engineering and one of the therapeutic strategies for patients with osteoporosis. The aim of this study was to assess the *in vivo* effects of LLLT on viability and calcium ion release of healthy bone marrow derived mesenchymal stem cells (BMMSCs) and osteoporotic-BMMSCs.

Materials and Methods: 18 female rats were divided into six groups. These groups included: 1. control healthy, 2. LLLT healthy, 3. control OVX, 4. LLLT-OVX, 5. Alendronate (Alen)-OVX, and 6. Alen+LLLT-OVX. Ovariectomy was done on rats of groups 3, 4, 5 and 6. After that all rats were euthanized and their MSC harvested and cultured in complete medium. In all groups, BMMSC viability, and calcium colorimetric assay were evaluated.

Results: We observed a significant increase in optical density(OD) of BMMSCs viability in LLLT healthy group compared to control-OVX, Alen-OVX, LLLT-OVX, LLLT+Alen- OVX, groups. LLLT+Alen-OVX group showed a significant increase in OD of BMMSCs viability compared to LLLT-OVX, Alen -OVX, and control-OVX groups. We observed a significant increase in calcium ion release of LLLT healthy group compared to control healthy, control OVX, Alen-OVX, LLLT- OVX, and LLLT+Alen- OVX groups. There were significant increases in calcium ion release of control healthy group compared to control OVX, Alen-OVX, LLLT- OVX, and LLLT+Alen-OVX groups. LLLT+Alen-OVX group showed a significant increase in calcium ion release compared to LLLT-OVX, Alen-OVX, and control OVX groups.

Conclusion: OVX-rats showed significant decrease in BMMSCs viability and calcium ion release compared to healthy group. This study showed biostimulatory effect of PWLLLT on viability and calcium ion release of healthy BMMSCs compared to Groups 3-6.

Keywords: Osteoporosis, Ovariectomy, Low-Level Laser Therapy

Ps-41: Cis pT231-Tau Is An Early Driver of Neurodegeneration in Bipolar I Disorder Examined Through Cellular Models

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Background: Bipolar disorder is an episodic recurrent pathological mood disturbance that ranges from extreme elation or mania to severe depression. Recent studies indicate that tauopathy may have contribution in pathogenesis of bipolar disorder. Lithium as a first-line treatment for bipolar disorder has been identified as an inhibitor of GSK-3 β which is one of the main kinases of tau protein. Also argyrophilic grains composed of phosphorylated tau have been observed in postmortem brains of bipolar patients. Furthermore, recent studies have demonstrated that phosphorylated tau at Thr231 exists in two distinct CIS and trans conformation in which CIS pT231-tau is highly neurotoxic and acts as an early driver of tauopathy in several neurodegenerative diseases. Although tau aggregation is detected in bipolar brain samples, its contribution to the disease etiology is not clear yet.

Materials and Methods: In this study we established cellular models of mania episode of bipolar disorder by overexpressing GSK-3 β in SH-SY5Y cells through transfection and examined cell viability, CIS p-tau and GSK-3 β expression in these models by immunofluorescence and Flow cytometry.

Results: We have found that CIS p-tau increased in mania model of bipolar disorder as viability decreased. Furthermore, we showed that lithium treatment inhibits CIS p-tau expression in these mania models.

Conclusion: This study shows that CIS p-tau may contribute to pathophysiology of bipolar disorders and could be the cause of neural cell death upon the disease which in turn would suggest novel therapeutic strategies against the disease.

Keywords: Bipolar Disorder, Cistauosis, GSK-3 β , Tauopathy

Ps-42: Design of A Novel 3-Layered NanoFibrous Mat for Wound Healing

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Background: The purpose of this study was to prepare and evaluate a 3- layered biomimetic applicable nanofibrous mat for wound dressing. The mat was composed of polycaprolactone (PCL) in the bottom layer, chitosan/poly ethylene oxide (Cs/PEO) in the middle layer and PCL/Collagen in the top layer.

Materials and Methods: Field emission scanning electron microscopy (FE-SEM) evaluation, mechanical properties and thermogravimetry analysis (TGA) were applied for characterization of nanofibrous mat. Then human dermal fibroblasts (HDFs) were seeded on the 3- layered nanofibrous mat and *in vitro* assessment was applied by using MTT and cell attachment with FE-SEM.

Results: HDFs could attach properly to surface of mat and showed normal growth and spreading. All three layers showed normal physical and mechanical characteristics that indicated integrity and strength of the three- layered nanofibrous mat.

Conclusion: This three- layered nanofibrous mat could be introduced as a dynamic and effective candidate for wound dressing.

Keywords: Wound Healing, Electrospinning, Scaffold, Three Dimensional Mat

Ps-43: Probable Molecular Mechanism for Restricted Adipogenic Differentiation Potential of Equine MSCs

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Background: It has been proposed that adipogenic potential of mesenchymal stem cells (MSCs) depends on transcription factors, age, hormones, growth factors, and donor. Adipogenesis is negatively regulated by multiple signaling pathway such as transforming growth factor β (TGF- β) and Wnt/ β -catenin signaling pathway. Adipogenic differentiation potential of equine adipose-derived MSCs (e-ADSCs) strongly depends on the components of the differentiation media, but the related mechanisms still are unclear. This study aimed to investigate the role of TGF- β 1 and CTNNB, as two key members of TGF- β and Wnt/ β -catenin signaling pathways on adipogenic differentiation of e-ADSCs.

Materials and Methods: e-ADSCs were isolated from the fat tissue of gluteal region and expanded *in vitro*. The cells at passages 5 were seeded and maintained in usual adipogenic culture medium. 75 % confluency of cells was considered as day 0 and cells were harvested at days 1, 3, 5 and 7. Then, total RNA was extracted and transcribed into cDNA using commercially available kits. Quantitative RT-PCR (qPCR) was performed using a SYBR green master mix and specific primers for GAPDH (as internal control), TGF- β 1 and CTNNB. Cells at day 0 were used as calibrator, data were normalized by GAPDH and analyzed using one way ANOVA. Adipogenic differentiation was assessed by Oil Red O specific staining.

Results: At the mRNA level, the expression of TGF- β 1 was significantly up regulated at days 3, 5 compared with day 1. Although mRNA level of TGF- β 1 was significantly reduced at day 7, but no significant difference was observed comparison to day 1. The expression of CTNNB were upregulated at all-time points compared with day 1.

Conclusion: The upregulated expression of both TGF- β 1 and CTNNB supports the view that TGF- β and Wnt signaling pathways may be one of the probable mechanisms involved in adipogenic inhibition of e-ADSCs.

Keywords: Adipogenesis, Mesenchymal Stem Cells, Equine, TGF- β , Wnt/ β -catenin

Ps-44: Investigating The Hepatic Differentiation of Induced Pluripotent Stem Cells by MiR-122 Upregulation and MiR-let-7f Downregulation

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Background: Induced pluripotent stem cells (iPSCs) can be a potentially matchless cellular resource for regenerative medicine because of their potential of differentiate into all kinds of cells and patient specific utilization. Since liver transplantation is the only accepted option for end-stage liver diseases, cell therapy by iPSCs can be a promising alternative way for orthotopic liver transplantation and improve the issue of the donor shortage and rejection risk. Although microRNAs (miRs) can act as powerful differentiation tool, few studies have been done in the field of iPSCs and hepatic differentiation by miRs. miR-122 is the most specific and abundant miR in the liver that play an important role in liver development. Another miR that

is important for hepatocyte differentiation is miR-let-7f, but in the reverse way; Therefore, let7-f downregulation (off-let-7f) hush hepatic differentiation.

Materials and Methods: iPSCs were transduced with lentiviruses containing miR-122, or off-let7-f, or negative control (scramble:scr). After making sure of miRs expression by qRT-PCR, hepatic differentiation was evaluated. Albumin (Alb), alpha fetoprotein (AFP), cytokeratin 18 (CK18), hepatic nuclear factor 4 α (HNF4 α) expression were analyzed by qRT-PCR. Alb and Urea secretion media were measured by photometric method. Glycogen storage was evaluated by Periodic acid-Schiff (PAS), and Alb, AFP, HNF4 α proteins were checked by Immunocytochemistry (ICC).

Results: It was shown that miR-122 upregulation and miR-let-7f downregulation could promote hepatic differentiation effectively. In the transduced groups, significant overexpression was detected for Alb, AFP, CK18, and HNF4 α and it had ascending trend with the most levels on days 14 and 21; Alb and Urea production enhanced significantly; Positive staining was detected for Alb, AFP, and HNF4 α on day 21, as well as for glycogen depositions.

Conclusion: Summing all these contents together indicate that using miRs for hepatic differentiation of iPSCs can be considered as a good candidate for improvement cell therapy for end-stage liver diseases.

Keywords: iPSCs, MiR-122, Let-7f, Hepatocyte

Ps-45: Anti-Cancer Effect of Hydro-Alcoholic Extract of Trifolium Pratense L. on U87MG Cell Line

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Background: Glioblastoma multiforme (GBM) is the most common type of the malignant astrocytic brain tumors. Natural products have played an important role in cancer therapy. The potential of using natural products as anti-cancer drugs was confirmed by international organization and nowadays there is a growing interest in discovery of naturally occurring anti-cancer drugs. Trifolium pratense L., a member of Leguminosae or Fabaceae family, is a short-lived biennial plant, which has been suggested for cancer treatment in traditional medicine. The aim of the present study was to investigate effects of T. pratense hydroalcoholic extract on a glioblastoma cell line (U87MG).

Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS. The effect of T. pratense extract (6.25, 12.5, 25, 50, 100, 200 and 400 μ g/mL) on cell viability was investigated using trypan blue staining, MTT assay, and lactate dehydrogenase activity measurement. Nitric oxide (NO) production was measured using Griess reaction. Data were analyzed by one-way ANOVA and P<0.05 was considered significant.

Results: Significant difference was seen among the groups treated with T. pratense extract compared to the control group (P<0.05) after 24, 48, and 72 hours. Increasing the dose significantly decreased cell viability (P<0.05). The IC50 values for 24-, 48- and 72-hours treatments were 398.37, 109.19 and 21.06 μ g/ml, respectively. Also, T. pratense extract significantly decreased NO production (P<0.05) by U87MG cells.

Conclusion: T. pratense extract reduced U87MG cell viability in dose- and time-dependent manner and showed anti-cancer

properties *in vitro*.

Keywords: Glioblastoma Multiforme, Natural Drugs, Nitric Oxide

Ps-46: Synergistic Effect of Trifolium Pratense Extract and Temozolomide on Glioblastoma Multiforme Cell Line

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Background: Glioblastoma multiforme (GBM) is the most malignant form of brain tumors and temozolomide (TMZ) is currently part of GBM standard treatment. Combination therapy can enhance the anti-cancer activity of TMZ. Trifolium pratense L showed an anti-cancer potential on some cell lines. The aim of the present study was to investigate the combination effect of T. pratense extract and TMZ on U87MG cell line.

Materials and Methods: The combination of TMZ and T. pratense extract was prepared in constant concentration ratio (5.57:1) based on their corresponding IC50 values in serial dilutions above and below the IC50 value of each agent, and then the MTT assay was performed. The combination index (CI) and dose reduction index (DRI) were calculated using CompuSyn software (ComboSyn, Inc., Paramus, NJ, USA). The CI values were interpreted as additive (CI=1), synergistic (CI<1) and antagonistic (CI>1). The DRI values represent the degree, to which the concentration of a compound can be reduced when used in combination with another compound, to maintain an equivalent effect. Finally, Fa is the fraction of cell death ranging from 0 (no cell killing) to 1 (100% cell killing).

Results: Cell viability reduction by TMZ and T. pratense extract combination was greater than either TMZ or T. pratense extract alone. In addition, CI and DRI values were calculated and showed that the CI values obtained in all tests were <1, indicating a synergistic effect. The DRI values for TMZ were >1 indicating a dose reduction for a given therapeutic effect.

Conclusion: Combination of TMZ and T. pratense extract had a synergistic cytotoxic effect.

Keywords: Drug Combination, Glioblastoma Multiforme, Temozolomide

Ps-47: Cis P-Tau Primes Amyloid Beta Aggregation; Studied In Vitro

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Background: Alzheimer disease is a progressive neurodegenerative disorder whose pathological hallmarks include amyloid plaques and neurofibrillary tangles composed of extracellular amyloid β (A β) and intracellular hyperphosphorylated tau respectively. It is of crucial importance to determine correlation between tauopathy and amyloidopathy. We herein study a causative link between A β and cis pT231-tau as the early driver of tauopathy.

Materials and Methods: Primary fetal rat hippocampal neurons underwent hypoxia (H₂O₂) for 96 hours. Then the cis pT231-tau and A β levels were observed at different time points. Furthermore, we examined A β levels in primary neurons upon cis p-tau administration.

Results: We have shown that Hypoxia culture of primary neurons induces cis pT231-tau sooner than A β aggregates. In contrast, A β does not show significant effect on cis pT231-tau accumulation.

Conclusion: Several attempts have been carried out to determine the exact interlink between A β and tauopathy but since they have considered the very late stages pathogenic p-tau epitopes, the approaches have not been that convincing thus far. We have shown that cis P tau appear first and trigger comprehensive destructions; such as A β aggregation. We believe our findings are indeed a remarkable advancement in field of AD research and may help us find an efficient therapeutic target.

Keywords: Beta Amyloid, Alzheimer's Disease, Cis pT231-Tau

Ps-48: Targeted Delivery of Medicinal Products in Advanced Therapy Using Cellular Knowledge Seeding

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Background: Advanced Therapy Medicinal Products (ATMPs) are innovative therapies that include gene therapy, somatic cell therapy, and tissue-engineered products. One of the important principle in the ATMPs is the delivery process of gene, cell, engineered tissue, and drug at the right time to the right place in the body. In order to handle delivery process especially to the critical organs e.g. brain that have Blood Brain Barrier (BBB), there is a great need for using intelligent delivery devices.

Materials and Methods: We introduce a swarm of nano carriers for collective detecting, and doing targeted delivery using cellular knowledge seeding which is defined in the synergy of artificial intelligence and biology. Here, each spot of the environment is a full laboratory for detecting disease marker, and the synergistic integration of spots' information will lead to a holistic diagnosis of the disease. Using online machine learning technique, this concept is implemented, and the carriers of the medicinal products using a swarm control are guided toward the inferred target.

Results: Result show the merits of the proposed method in targeted cancer drug delivery by decreasing apoptotic cell density, and hypoxic cell density which are the representation of cancer curing.

Conclusion: ATMPs as innovative therapies provide a wide range of applications in the case that normal therapies are not effective. ATMPs use carriers for medicinal products delivery. This autonomous navigation use collective knowledge of the disease microenvironment for detection and therapy. In this research swarm of these carriers are used for targeted delivery.

Keywords: Targeted Delivery, Advanced Therapy Medicinal Products, Knowledge Seeding, Artificial Intelligence

Ps-49: Advanced Therapy and Cognitive Science: Structure and Infrastructure for Having Effective Collaborative Therapy

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Background: Innovative therapies e.g. gene therapy, somatic cell therapy, tissue-engineered products, and target drug delivery are all members of a great family of Advanced Therapy Medicinal Products (ATMPs). In order to make the structure of ATMPs more effective, there is a need for a proper infrastructure in which the cellular, tissue, and organ processes smooth and coordinate their work. Studies show that brain, mind, and its cognitive activities can be considered as such infrastructure.

Materials and Methods: In this study, the effect of cognitive science on having more effective advance therapy is discussed. ATMPs which is presented at different scale can be more effective when it is in the presence of the brain, and mind coordination. Here, we discuss the effect of several cognitive method such as coping and adaptation by the help of cognitive process of having positive thinking and empathy, having adequate sleep, ordering cognitive impairment, cognitive rehabilitation, mind body therapies (MBT), and finally we discuss the effect of stem cell therapy on the cognitive functions.

Results: Result show that ATMPs can be more effective when it is in the presence of the brain and mind coordination. In other words, cognitive process paves the way for having more effective advanced therapy. It has positive effect on cancer curing, changing the sympathetic nervous system activation of gene transcription factors which are involved in immune function and inflammation, and also it can be considered as a pre-surgical treatment.

Conclusion: ATMPs as innovative therapies need to be accompanied with a therapeutic framework that uses both body and mind. Here, the intersection of ATMPs and cognitive sciences is studied in order to reach an effective therapy.

Keywords: Collaborative Therapy, Cognitive Science, Advanced Therapy Medicinal Products

Ps-50: Fabrication of Polypyrrole Coated Electrically Conductive Nanofibers for Tissue Engineering Applications

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Background: It has been demonstrated that Electrical stimulation can enhance cellular behaviors such as proliferation, migration, elongation and even differentiation especially in electrically active cells such as neural and muscle cells. To this end, electrically conductive polymers have been explored to facilitate direct electrical stimulation of cells. Meanwhile, electrospinning method has been widely used to produce nanofibrous scaffolds capable of providing anchorage sites for cells to adhere while allowing transport of metabolites. In this study we demonstrate how an electrically conductive nanofibrous scaffold can be attained by polymerization of pyrrole (PY) monomers on the surface of an electrospun base scaffold.

Materials and Methods: The base scaffold was prepared by electrospinning of a poly-L-lactide (PLLA) solution in a mixed solvent of chloroform (CHL) and dimethylformamide (DMF). Then the base scaffold was dipped in an aqueous solution of PY and the oxidant was added to initiate the polymerization reaction. To enhance the quality of coating, the reaction was slowed by lowering the temperature to 4°C and gentle stirring was applied overnight. To increase hydrophilicity and activate the surface, vacuum air plasma was used both prior and after pyrrole deposition to obtain more uniform coating and enhanced cellular attachment. Morphological features of the scaffold before and after coating were examined using scanning electron microscopy (SEM). The depth of the coated layer was derived using the change in mean fiber diameter due to the coating procedure. Chemical bonds of Polypyrrole in the coated scaffold were verified by Fourier-transform infrared (FTIR) spectroscopy using attenuated total reflectance (ATR) technique. To obtain hydrophilicity, water droplet contact angle assay was performed. To assess electrical conductivity, two parallel electrodes were placed at a fixed distance and a gradual increase in voltage was applied. The slope of the I-V plot was used to calculated conductivity.

Results: SEM images revealed that the best morphology was obtained when a 9%w/v PLLA was dissolved in a 17:3 CHL to DMF mixture. For the coating stage a solution of 10mM PY with a 1-1 molar ratio of ammonium persulfate as the oxidant was selected. Significantly higher concentrations lead to filling of the pores while lower concentrations can result in sparse and inadequate coating, however, the conductivity of the scaffold can still be tailored by changing the depth of the coated layer which in turn can be controlled by tuning the monomer and oxidant concentration near the selected value. Electrical conductivity of around 60mS.m was measured for the final scaffold. Multiple rounds of coating can be applied if a significant increase in conductivity is required for other applications. To measure contact angel, a 6µl droplet was placed on the scaffold and the angle was measured 5s after contact. Angles of 127° and 120° was measured for the base and the coated scaffolds respectively, while complete absorption of water into the lattice of both scaffolds were observed after plasma treatment.

Conclusion: In conclusion a nano-fibrous electrically conductive scaffold was introduced. This scaffold is capable of applying electrical stimulation to cells directly, eliminating the need for a metal electrode to be in direct contact with culture medium, thus phasing out the possibility of electrode toxicity. The plasma treatment can lead to higher cellular attachment due to the increased hydrophilicity of the surface.

Keywords: Eclectically Conductive Nano-Fibrous Scaffold, Scaffold for Electrical Stimulation of Cell, Polypyrrole Coated Poly-L-Lactide Nanofiber

Ps-51: Evaluation of Gene Expression and Methylation of DOK7 Gene in Blood Samples of Patient with Breast Cancer

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Background: Breast cancer is one of the most common cancer among women. Due to lack of the highly specific biomarkers of

carcinogenesis and prognostic evaluation, the overall survival of breast cancer is still dismal. DOK7 is type of genes which involves in neuromuscular synapses. Researchers reported that the expression and methylation of DOK7 as a tumor suppressor. Also they demonstrated methylation of DOK7 as an indicator of breast cancer risk that overexpression correlated with patient survival. The aim of this study was to evaluate the expression and methylation of DOK7 gene in breast cancer patients compared to normal patients and to evaluate its role as a prognostic and diagnostic factor in breast cancer and to investigate the possible relationship between DOK7 gene expression variation and age and methylation status.

Materials and Methods: In this study, 80 samples of venous blood were prepared from Farda's lab to determine the gene expression and the status of promoter methylation. The samples were from the year 1395. The expression of DOK7 gene was investigated by Real-Time PCR and DOK7 promoter methylation was investigated by MS PCR.

Results: In this study, we found that the expression of DOK7 was decreased in patients' blood samples compared with normal blood samples and the reduced expression is associated with promoter methylation and also the results of DOK7 promoter methylation showed that 72.5% of promoter in patients' blood samples was methylated and 20% of promoter in normal blood samples was unmethylated.

Conclusion: The reduced expression of DOK7 and DOK7 promoter methylation in patients' blood samples would be a candidate biomarker for breast cancer prognosis.

Keywords: Breast Cancer, Real-Time PCR, MS PCR, DOK7

Ps-52: Chemotactic Activities of Human Bone Marrow Mesenchymal Stem Cells Was Inhibited Following Incubation with Diabetes Mellitus Type II Serum

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Background: Type 2 diabetes mellitus (DM2) are susceptible to progress associated cardiovascular disease, abnormal angiogenesis, and vascular bed complications. It was previously revealed that specific bone marrow stem/progenitor populations were more sensitive to a persistent diabetic condition which associated with numerous fundamental defects. Accumulating data support the hypothesis that bone marrow mesenchymal stem cells (BM-MSCs) could migrate and home to the wound area to promote angiogenesis by various mechanisms. We investigated the effect of DM2 sera on chemotactic activities of human bone marrow mesenchymal stem cells *in vitro*

Materials and Methods: BM-MSCs were allocated into three groups; control (DMEM with 10% FBS); non-diabetic (DMEM with 10% normal human serum), and diabetic (DMEM with 10% diabetic serum) groups. After 7 days treatment, we performed an *in vitro* migration assay by using 8- μ m pore size Transwell membrane inserts. BM-MSCs were resuspended in

200 μ l DMEM containing 2% diabetic or non-diabetic serum and transferred into the Transwell insert. In control group, cells were only exposed to 2% FBS. In the lower basolateral chamber, 700 μ l of DMEM enriched with 50 nM SDF1- α or 10 ng/ml VEGF was added. After incubation at 37°C for 24 h, the number of migrated cells was counted in 5 random high-power fields. In order to verify the results of migration/chemotactic assay, the expression of CXCR-4, VEGFR-2, and VEGF were quantified by real-time PCR system with the SYBR Green PCR Master Mix. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at P<0.05.

Results: In factor free condition, the number of migrated BM-MSCs after exposure to diabetic condition was significantly diminished as compared with non-diabetic and control groups (pdiabetic sera versus control<0.0001; pdiabetic versus non-diabetic sera<0.05). Similar to factor-free condition, a marked reduction was observed in diabetic BM-MSCs in response to SDF-1 α (pdiabetic versus control <0.01; pdiabetic versus non-diabetic groups<0.05). Compared with either control or non-diabetic groups, diabetic sera abolished the migration of BM-MSCs in response to VEGF (pdiabetic sera versus control and non-diabetic groups<0.0001). Compared to control group, the mRNA level of CXCR-4 gene in non-diabetic and diabetic group was significantly reduced (pcontrol versus diabetic and non-diabetic sera<0.001). Similar to expression pattern of CXCR-4, DM2 had potential to reduce the expression of VEGFR-2 (pdiabetic sera versus control<0.05; pdiabetic sera versus non-diabetic sera<0.01) and VEGF (pdiabetic sera versus control<0.001; pdiabetic versus non-diabetic sera<0.01) as compared with control and non-diabetic groups.

Conclusion: Our data revealed that DM2 condition diminished chemotactic activity of BM-MSCs toward VEGF and SDF-1 α and also DM2 sera could suppress SDF-1 α /CXCR-4 and VEGF/VEGFR-2 axis in BM-MSCs.

Keywords: Diabetes Type II, Mesenchymal Stem Cells, Chemotactic, Migration

Ps-53: Diabetes Mellitus Type 2 Sera Increased Exosome Secretion Pathway Activity in Human Marrow Mesenchymal Stem Cell *In Vitro*

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Background: Exosomes, nano-sized cell-derived membrane vesicles (30-120 nm), secreted by many cell types. It was recently demonstrated that exosomes contribute in cell-to-cell communication by transfer of bio-materials. Exosomes participate in physiological and pathological processes. Previous studies described adverse effects of diabetes mellitus type 2 (DM2) on stem cell function and biology. It was revealed that human marrow mesenchymal stem cells (hMSCs) were more sensitive to a persistent diabetic condition which associated with changes in their secretome. In the present study we aimed to study the

effect of serum from type 2 diabetic mellitus patients on the exosome secretion pathway in human mesenchymal stem cells *in vitro*.

Materials and Methods: For *in vitro* assays, hMSCs were classified into two groups as follows; Control: cells received DMEM/LG and 10% sera from healthy subjects; Diabetic groups treated with DMEM/LG and 10% diabetic sera over a period of 7 days. To investigate expression of Alix, CD63, Rab27a, Rab27b, and Rab8b genes, we performed real-time PCR (Rotor-Gene 3000, Corbett Robotics) using SYBR Green PCR Master Mix (Cat no: YT2551, Iran). To further confirmation of CD63 level, protein level of CD63 was examined using western blotting method and densitometry analysis was performed with ImageJ software ver.1.44p (NIH). Furthermore, exosome secretion quantified by acetylcholinesterase (ACE) assay using kit (Cat No: BXC0801; Biorexfars). Choline esterase activity was calculated by following formula; Activity (U/l) = $\Delta\text{Abs}/\text{min} \times 65800$. Data are expressed as mean \pm SD. Student's t-test was used to calculate the significance of differences between groups. Values of $P < 0.05$ were considered statistically significant.

Results: Compared to control group, the mRNA level of Alix, CD63, Rab27a, and Rab8b genes in diabetic group was significantly increased (pcontrol versus diabetic < 0.01). Furthermore, mRNA level of Rab27b was enhanced as compared to control group ($P < 0.05$). Western blotting analysis showed that the protein level of CD63 was significantly enhanced in diabetic group (pcontrol versus diabetic < 0.01). Acetylcholinesterase activity was significantly increased in diabetic group (pcontrol versus diabetic < 0.001).

Conclusion: The present study provides the evidence of a specific mechanism that stem cell biology was altered through exosome signaling pathway in diabetic mellitus. As a result, we consider diabetic hMSC release more exosomes through upregulation of genes involved in exosome secretion pathway.

Keywords: Exosome, Human Marrow Mesenchymal Stem Cell, Diabetes Mellitus, Rab

Ps-54: A Discovery to Figure out Toxicity Mechanism in TBI Models

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Background: Traumatic brain injury (TBI) has been suggested as the biggest risk factor for chronic neurodegenerative disorders; such as Alzheimer's disease and amyotrophic lateral sclerosis. It has been shown that phosphorylated tau at Thr231 in the cis conformation is the central mediator of cell death upon tauopathy. Besides, cis p-tau is being considered as the earliest tau epitope; ahead of any oligomer, aggregate and tangle epitopes, resulting in cell death. Also, neurons treated with TBI brain lysates had much higher rate of apoptosis. Thus, cis p-tau is indeed neurotoxic and plays key role in neurodegeneration upon tauopathy and TBI.

Materials and Methods: To test the hypothesis, TBI mouse brains were immunostained with cis p-tau.

Results: We have found that neurotoxic cis p-tau initially moves

into nucleus in the TBI brains; causing cell death in a time dependent manner. Thus, we hypothesized that cis p-T231-tau is becoming neurotoxic upon its nuclear translocation. To test the hypothesis, TBI mouse brains were immunostained with cis p-tau. Our results demonstrated that the neurotoxic cis, but not trans, pT231-tau moves into nucleus. Furthermore, cis p-tau plays its pathogenic roles upon its translocation.

Conclusion: Taking these together, we concluded that cis p-tau kills the cells through some physiological processes interruption; likely by interacting with nuclear factors. Our data open new windows toward understanding tauopathy and TBI molecular mechanisms and further may shed light to possible treatments for neurodegenerative disorders.

Keywords: TBI, Tauopathy, Neurodegeneration, Cis p-tau

Ps-55: Effects of Autologous Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells on Arterial Blood Gas Pressure in Experimental Model of Acute Respiratory Distress Syndrome in Sheep

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Background: Acute respiratory distress syndrome (ARDS) is a lung disorder that causes death in human and animals. Due to the high prevalence of pulmonary disease worldwide, developing preventive therapies to reduce lung disease are important. There is no particular therapeutic drug for ARDS. For this reason, achieving a new therapeutic approach, such as stem cell therapy, is a necessary requirement. The use of mesenchymal stem cells for the treatment of pulmonary injuries has become a promising therapeutic approach.

Materials and Methods: Ten male sheep were used after random placement into two groups. In the treatment group bone marrow samples were collected and BM-MSCs isolated and amplified. Then an experimental model of ARDS was induced by intratracheal injection of LPS. Radiograph images performed before and after injection of LPS. After confirming inflammation and 50×10^6 cells of BM-MSCs were transplanted in the treatment group as autolog. An arterial blood sample was used for analysis of blood gases. The arterial samples were collected from the ear artery in the before and 3, 6, 12, 24, 48, 72 and 168 hours after BM-MSC transplantation or PBS injection and the sample were processed with Blood Gas Analyzer, immediately. Blood gas analyzer measured pH, the partial pressure of oxygen (PO₂), the partial pressure of carbon dioxide (PCO₂) and bicarbonate (HCO₃) on arterial blood samples.

Results: The results showed that stem cell transplantation could modulate hypoxemia in inflammation, unlike the control group. Arterial blood gas analysis was improved in lung injury following MSC treatment. The survey of the PO₂ showed a significant decrease in the two groups after creating an experimental

model of ARDS. But after the BM-MSCs transplantation PO₂ increased, so that it was significant at times 24, 48, 72 and 168 h comparison inflammation time. Also, decrement in PCO₂ occurred after the BM-MSCs transplantation, so that the reduction was significant at times of 24, 48, 72 h compared with baseline.

Conclusion: The results indicated that BM-MSCs could improve arterial blood gas and play a significant role in the repair of ARDS.

Keywords: BM-MSCs, ARDS, Arterial Blood Gases, Sheep

Ps-56: Generation of Functional Liver Organoid by Co-Culture of Human Hepatocellular Carcinoma Cells with Non-parenchymal Cells in Collagen Hydrogel

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Background: Advances in stem cell technology, tissue engineering and regenerative medicine have enabled the generation of three-dimensional (3D) organoid culture. Diverse types of organoid have been developed that exhibit multiple cell types and cell function, properties that were difficult to achieve with custom 2D culture systems. Previously takebe et al, demonstrated that interactions between hepatic parenchymal and non-parenchymal cells key to advance self-organizing liver bud. On the other hand, collagen type I (Col), as major liver extracellular matrix (ECM) components, has typically been used for hepatocyte culture and transplantation. Collagen as a natural microenvironment can govern cell expansion and organoid formation.

Materials and Methods: In this study we developed a liver organoid (ColGel-organoid) using co-culture of endothelial, mesenchymal and hepatocarcinoma cell line (Huh7) within collagen hydrogel (ColGel). We showed the presence of non-parenchymal cells beside an appropriate ECM component (collagen) is critical to generate functional liver organoid.

Results: We found ColGel was sufficient for cell survival. Furthermore, the ColGel together with non-parenchymal cells provide essential supportive and inductive matrix for hepatic specific functions in Huh7 cells. The ColGel-organoid showed up-regulated mRNA levels of multiple hepatocyte genes, specially expression of CYP3A4 and 3A7 increased more than 100 times in comparison to 2D culture of Huh7. ColGel-organoids showed elevated ALB and AAT secretion, urea synthesis and CYP3A4 enzyme activity and inducibility in comparison to collagen free organoid.

Conclusion: In summary, the generated ColGel-organoid could effectively promote the Huh7 cell functionality *in vitro*; so this method is an appropriate approach for three-dimensional liver

organoid culture. It is suggested that the ColGel-organoid could be used as a novel *in vitro* model system for basic and clinical research of liver.

Keywords: 3D Liver Organoid, Collagen Hydrogel, Non-Parenchymal Cells, Tissue Engineering, Regenerative Medicine

Ps-57: The Effect of Co-Culturing Human Adipose-Derived Stem Cells with Rat Retinal Cells Survival and Axonal growth

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Background: Retinal nerve cells have limited regeneration ability the same as the central nervous system. This regeneration deficiency is usually attributed to the myelin structure and glial scar. If the axonal processes are impaired for any reason, their regeneration to reestablish the connection with different parts of brain will not be possible. However, small axon-like shoots may appear from these cells near the impaired area. There are various strategies for retinal regeneration, including gene therapy, laser therapy and use of stem cells. Human adipose-derived stem cells (hADSCs) are able to differentiate into different cells and secrete various neurotrophic factors that affect other cells. So far, no study has investigated the effect of hADSCs on retinal cells. The present study was aimed to investigate the effect of hADSCs and their secretory environment on the axonal survival and growth of retinal cells *in vitro*.

Materials and Methods: The hADSCs of patients candidate for surgery were isolated, cultured and passaged. The rat's retina, after isolation, was placed in closed containers. After preparing the supernatant of hADSCs, retina along with the cells (Retin/hADSC) as well as their supernatant (Retin/CM) with PDL/ laminin (Poly-D-Lysine/Laminin) were co-cultured for 24 hours. The survival rate was assessed by MTT and IHC methods at days 1, 7 and 14. The axonal growth was determined by immunofluorescent technique and β -tub III antibody. Then, axonal growth was analyzed by ImageJ software at day 14 in retinal cells.

Results: In direct co-culture group (Retin+hADS), a significant difference was found in the survival of retinal cells at day 14 in comparison with day 7 ($P < 0.01$) and day 1 ($P < 0.001$). In indirect co-culture group (Retin+CM), a significant difference was observed in the survival of retinal cells at day 14 compared to days 1 and 7 ($P < 0.001$). The analysis of axonal growth in retinal cells, Auto-analysis of the number of intersections (number of axonal processes) and distance of axonal processes as well as comparison of control (Retin), direct co-culture (Retin+hADSC) and indirect co-culture (Retin+CM) groups showed the number of intersections was significantly higher in direct and indirect co-culture groups than control group ($P < 0.05$), while the number of intersections was significantly higher in indirect co-culture group than direct co-culture group ($P < 0.05$). The results of Auto-analysis by ImageJ software indicated the number of axonal deformities was significantly higher in indirect co-culture group than control group ($P < 0.01$).

Conclusion: hADSCs along with their supernatant can increase the axonal survival and growth in retinal cells. Given the secre-

tion of different factors by hADSCs and their significant effect on the nerve cells, the results of this study can help to develop basic methods for the treatment of eye diseases in the future.

Keywords: hADSCs, Supernatant, Retina, Co-Culture

Ps-58: *In Vitro* Assessment of Aligned Electrospun Poly (Glycerol Sebacate)/Poly (Vinyl Alcohol) Nanofibrous for Peripheral Nervous Tissue

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Background: Peripheral nervous tissue damage is relatively common which could rise from tumor resection, infections (e.g., Lyme disease), systemic diseases (e.g., diabetes), and trauma. Electrospinning of synthetic polymers is one of the most practical approaches for using in tissue engineering. Electrospinning of PGS is challenging due to the low molecular weight of PGS, and it's necessary to use a carrier polymer for electrospinning this polymer. Since aligned fibers result in promoting neural cell growth and guiding targeted axon propagation, aligned fibers were fabricated in this experiment.

Materials and Methods: In this study, poly (vinyl alcohol) was chosen as carrier polymer and then aligned PVA-PGS scaffold was fabricated by electrospinning technique. Morphology and microstructure assessment of PVA-PGS fibers were investigated by scanning electron microscopy (SEM), mechanical properties of the scaffold were determined by the universal testing machine, and chemical analysis of scaffold was also studied by Fourier-transform infrared (FTIR) for confirming successful blending. To assess the biocompatibility of scaffold, the PC12 neuronal-like cell has been cultured on fabricated fibers for 7 days.

Results: The result showed that produced fibers had smooth and bead-free morphology with a uniform fiber diameter in nanoscale. Young's modulus of PVA-PGA scaffold was close to nervous tissue. MTT assay also showed good biocompatibility of fabricated scaffold.

Conclusion: Our results suggest that PVA-PGS is a suitable and promising scaffold for peripheral nerve regeneration.

Keywords: Peripheral Nerve Regeneration, Electrospinning, Poly(glycerol sebacate), Poly(vinyl alcohol), Nanofibrous

Ps-59: Fabrication of Aligned Electrospun Polycaprolactone-Lignin Nanofibers for Peripheral Nervous Regeneration

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Background: Damage to peripheral nervous tissue results in pain and significant disability due to disorder sensory and motor functions in the body. On the other hand, the structure and function complexity of the nervous system cause complications

and challenges in peripheral nerve regeneration. Among several methods for treating nerve damage, tissue engineering has emerged as a novel approach to the rapid treatment of damaged nerve tissue. Electrospinning technique is an effective technology to fabricate aligned fibrous membrane for stimulating the Extracellular Matrix (ECM) in the nervous system.

Materials and Methods: In this study, electrospun polycaprolactone (PCL) and lignin fibers were fabricated and characterized by SEM, contact angle and Fourier-transfer infrared (FTIR). Attachment and viability of PC12 cells on PCL/lignin scaffold were evaluated by SEM and MTT assay.

Results: The morphology and structure of fibers showed that the fabricated fibers were oriented, bead-free, and in nanoscale size. The resulting data indicated that lignin can significantly improve the hydrophilicity properties of the scaffold. Attachment and viability of PC12 cells on PCL/lignin scaffold were evaluated by SEM and MTT assay, which showed biocompatibility of PCL/lignin scaffold.

Conclusion: Our research indicates that electrospun PCL/lignin has good potential for nerve regeneration.

Keywords: Peripheral Nerve Regeneration, Electrospinning, Nanofiber Scaffold

Ps-60: Decrease in The Telomere Length of Bone Marrow Mesenchymal Stem Cells Co-Cultured with K562 Cell Line Along with A Change in The Expression Level of WNT/B-Catenin Proteins

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by a proliferation of myeloid cell lineage and chromosome translocation t(9;22), so-called Philadelphia chromosome. Without effective therapy, CML progresses in three successive phases: chronic (CP), accelerated (AP), and blast crisis (BP). As is the case in all cancers, telomeres play an important role in the progression of CML. Telomere shortening has been reported in each of the three phases of CML, and this shortening is accentuated during progression of the disease. On the other hand, cell transplantation with bone marrow derived mesenchymal stem cells (BMSCs) is one of the central therapeutic treatments for hematologic cancers. The assessment of individual telomere length profiles in CML will provide knowledge concerning specific individual telomere length changes associated with CML. Therefore, the aim of this study is investigation of the effect of BMSCs on telomere length of co-cultured K562 cell line via Wnt/ β -catenin signaling pathway.

Materials and Methods: In this study, the bone marrow was flushed from the femur of *rattus norvegicus*. Next, mononuclear cells were separated by ficoll hypaque and bone marrow derived mesenchymal stem cells (BMSCs) were isolated. In addition, immunocytochemistry staining and flow cytometric analysis were performed to investigate the MSCs-surface markers.

Subsequently, K562 as chronic myeloid leukemia cell line were cultured in RPMI/1640. After reaching confluency of cells, BMSCs co-cultured with K562 cell line for 7 days (1:10). At the end of co-culture time, K562 cell line was collected, DNA and protein were extracted and subjected to Real-time PCR and western blotting, respectively. Quantitative real-time PCR and western blotting were used to measure the absolute telomere length and Wnt/ β -catenin protein expression, respectively.

Results: It was found that BMSCs had the capacity to adhere to culture plastic flasks. Also, immunocytochemistry staining and flow-cytometric analysis showed that BMSCs had high levels of expression of CD44 (94.5%) and CD90 (87.1%) and hematopoietic cell lineage-specific antigens, such as CD31 (0.07%), and CD56 (0.9%) were not expressed in these cells. In addition, quantitative real-time PCR showed that BMSCs cause to decrease telomere length of K562 cell line significantly. Along with these results, evaluation of Wnt/ β -catenin protein expression indicated the decreased in level of both proteins.

Conclusion: Taken together, the data indicated that changes induced in the absolute telomere length of K562 co-cultured with BMSCs via Wnt/ β -catenin signaling pathway.

Keywords: Absolute Telomere Length, Bone Marrow-Derived Mesenchymal Stem Cell, Wnt/ β -Catenin Signaling Pathway

Ps-61: Fabrication and Characterization of ZnO-Nanoparticles, Using as siRNA Delivery Reagent in Prostate Cancer

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Background: Great conflict in the treatment of prostate cancer tends to castration-resistant prostate cancer cases. Unfortunately, most of the patients experience some degree of androgen independency even after two years of starting routine treatments. Recent developments in personalized medicine in advanced cancer treatment, opened new windows in to survey whom struggling with prostate cancer, particularly castration-resistant patients. Nowadays, a great deal of attention directed towards specialized treatments which focus on interacting the genes whose activity tends to grow in cancer. Using siRNAs capable the scientists to manipulate the gene activity. Due to siRNA instability, its widespread use as systemic treatment in cancer patients is still need more overview. On the other hand, for using the siRNAs as treatment reagents, must be ensured to be released only in the target tissues. The capability of nanoparticles in carrying and releasing drugs in target organs motivated the hypothesis in which siRNAs will be stabilized in conjunction with nanoparticles and then it would be expected to be gradually released in the target tissues. In the view of the fact that Metal concentration of zinc in prostate cells is 800-1600 times greater than in other body cells, applying zinc nanoparticles to carry and deliver drugs to the target tissue appears to be a reliable method.

Materials and Methods: Zinc oxide nanoparticles were synthesized by sol-gel method using zinc acetate and methanol as precursors. In the preparation, 16 g of zinc acetate was dissolved in 112 ml of methanol. After 10 minutes magnetic stirring at room temperature. Nano zinc oxide was washed several times with

double distilled water to remove the byproducts. After washing, the ZnO nanoparticles were dried at 80°C in hot air oven with constant stirring for 5 hours. The resultant powder was annealed at 500°C for 5 hours. The final product ZnO nanoparticles were investigated by X-Ray Diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), Scanning Electron Microscope (SEM), and Cell viability was measured after 3, 5 and 7 days using the MTS assay in PC3M cells-highly malignant prostate cancer cell lines (for minimum toxicity to healthy cells from ZnO without conjugated drug) respectively.

Results: Results from recent study shows a typical XRD pattern of ZnO nanoparticles, the average crystal size of synthesized nanoparticles was calculated and FT-IR peak at 417.52 cm⁻¹ indicated characteristic absorption bands OF ZnO nanoparticles. The SEM image shows that ZnO nanoparticles prepared in this study are spherical in shape. Using MTS assay, it has been shown that PC3M cell viability were intact when treated ZnO with a concentration of 35 μ g/ml. On the other hand, Forootan, et al studies revealed that delivering siRNAs by atelocollagen could reduce the prostate cancer progression in mice model.

Conclusion: Up to the finding of this study we decided to make zinc nanoparticles and charge them with siRNA with the aim of applying them to cancerous cells. This study focused on fabrication and characterization ZnO-NPs for the biomedical applications and drug delivery to improve their targeting and cytotoxicity against cancer cells and minimum toxicity to healthy cells.

Keywords: ZnO-Nanoparticles, siRNA Delivery, Prostate Cancer

Ps-62: In Vivo Tumourigenicity of A Genetic Abnormality Frequently Observed in Human Embryonic Stem Cells in A Differentiated Cell Engraftment Model

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Background: Pluripotent stem cells can be cultured indefinitely, however, during expansion; cells were susceptible to chromosomal aberrations and karyotype abnormalities. Screening of 136 stem cell lines for genetic changes revealed a minimal amplicon in chromosome 20q11.21 containing BCL2L1, an anti-apoptotic gene, in 20% of cell lines.

Materials and Methods: Four clones of ES cells were selected on the basis of +/- 20q.11.21, stably-transfected with an enhanced luciferase gene. Clones were differentiated into hepatocyte-like cells (HLCs) and 1 \times 10⁶ HLCs (plus one vehicle control) were administered into the spleen of mice, three days after an acute administration of carbon tetrachloride. Live imaging was performed on a weekly basis over 14 weeks. Liver and spleen were examined histologically.

Results: Function pluripotency of ES cells to all three germ layer were confirmed using embryoid body formation. Bioluminescence signals were significantly higher in +20q.11.21 ES cells compare to -20q.11.21. Extend of liver mass and histol-

ogy of liver tissue from mice received ES cells with amplicon 20q.11.21 were significantly different to the mice received cells without the amplicon.

Conclusion: Presence of amplicon 20q.11.21 in stem cells can predispose cells to tumour formation and screening for the amplicon is very important before any clinical application.

Keywords: *In Vivo* Tumorigenicity, Genetic Abnormality, Human Embryonic Stem Cell, Chromosome 20q11.21, Bioluminescence

Ps-63: MicroRNA-218 Can Differentiate Mesenchymal Stem Cells to Osteoblast without Osteogenic Media Supplements

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Background: Osteoporosis is a disease marked by reduced bone mass, leading to an increased risk of fractures or broken bones. Elucidation of the molecular mechanisms which regulate mesenchymal stem cell differentiation into osteoblasts, is of great importance for the development of anabolic therapies for osteoporosis and other bone metabolism-related diseases. microRNAs (miRNAs) have crucial roles in bone development, osteogenic differentiation, and osteoporosis pathophysiology. Wnt/ β -catenin signaling activity is also critical in osteogenesis and regulated by miRNAs. It had been shown that miR-218 was significantly upregulated during osteogenic differentiation in human adipose derived MSCs (hAMSCs) and directly targets sFRP2, DKK2, and also Sclerostin which is a WNT signaling pathway antagonist.

Materials and Methods: The pre-miRNA nucleotide sequences of miR-218 was cloned into pEGP-MIR vector (Cell Biolabs, Inc). hAMSC were isolated, culture in proper media and characterized by flowcytometry. A total of 1 pEG-MIR-218 containing or empty plasmid was transfected into hAMSCs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The osteogenic differentiation was followed in groups for 21 days.

Results: During osteogenic differentiation, a significantly higher values of alkaline phosphatase activity, mineralization, and osteogenesis-related gene expression was observed in hAMSC transfected with pEGP-miR-218 compared to pEGP-control in conditional medium.

Conclusion: The results demonstrated that targeting antagonist of Wnt/ β -catenin signaling pathway will strengthen osteogenic differentiation of hAMSC even without differentiation medium. Thus, our findings suggest that miR-218 therapy may serve as a novel therapeutic agent for treatment of osteoporosis and other bone metabolism-related diseases.

Keywords: Osteogenesis, MiR-218, Mesenchymal Stem Cell

Ps-64: Neuroprotective Effects of Intravenous Injection of Human Embryonic Stem Cell-Neural Progenitor Cells in Traumatic Optic Neuropathy Mouse Model

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Background: Retinal ganglion cells (RGCs) take signals coming from visual stimuli in the eye and deliver them to the brain's visual cortex through their axons in the optic nerve. Any damage to the optic nerve would lead to the degeneration of non-regenerating RGC axons and death of irreplaceable RGCs. Optic nerve damage can lead to a total loss of vision, a permanent organ damage that in most cases is not curable through surgery or medication. Neuroprotective functions of stem cells in the nervous system have prompted many studies investigating the effectiveness of a range of these cells on various retinal disease models. Neural progenitor cells (NPCs) secrete an assortment of trophic factors that are vital to the protection of the visual system.

Materials and Methods: Three animal groups were used for comparison: 1. Healthy, 2. Vehicle, and 3. NPCs. These behavioral groups were compared using the Visual Cliff behavioral test, immunohistoflourscent (IHF) staining using Brn3a marker for RGCs and GFAP for astrocytes, retrograde tracing with DiI injection into the superior colliculus, and study Retinal layer thickness using H&E staining.

Results: Studying the effects of 50.000 NPCs intravenous injection through visual cliff showed any vision improvement in NPCs compared to the vehicle group. In contrast, retrograde tracing test and IHF staining of the RGCs revealed their higher RGCs concentration in NPCs group. Moreover study of Retinal thickness showed more thickness in NPCs group compared to vehicle group. Most of the tests indicating that the NPCs protect RGCs from degeneration.

Conclusion: Due to ability of NPCs to secrete trophic factors, they may be used for optic nerve protection.

Keywords: Neural Progenitor Cells, Traumatic Optic Neuropathy, Neuroprotection, Retinal Ganglion Cells

Ps-65: The Effect of Maternal Sleep Deprivation on Differentiation of Mesenchymal Stem Cells in The Presence of Neonates Brain CSF of Wistar Rats

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Background: Cerebrospinal fluid (CSF) contains many neurotrophic and growth factors. Due to presence of such factors this fluid has proliferative and differentiation potential. Maternal sleep deprivation (MSD) decrease number of newborn neurons in development of hippocampus. Also, impairs hippocampus-dependent spatial learning and memory in the young offspring

rat. Because, MSD can change CSF factors, thus in the present study, the effect of MSD on differentiation of mesenchymal stem cells in the presence of neonates brain CSF was examined. **Materials and Methods:** In this study, bone marrow stem cells were aspirated from the femur and tibia of young male rats. Then cell suspension cultured in DMEM medium supplemented with FBS and antibiotic. CSF was collected from cisterna magna of neonates rats. CSF added to culture media with 10% ratio (v/v). Cell viability determined with MTT assay. Total cellular RNA was extracted and cDNA was synthesized and NeuN, Nestin, Neurod1 genes analyzed.

Results: RT-PCR analyze shows that neurogenesis decrease significantly with a little function compares to control group.

Conclusion: Based on our results, MSD decrease neurogenesis in mesenchymal stem cells, compared to control group. It seems that MSD can change CSF factors.

Keywords: Sleep Deprivation, CSF, Stem Cells

Ps-66: An Investigation of Promoter-Targeted Small Activating RNAs on BMP2 Up-Regulation

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Background: saRNAs (Small activating RNAs) are small double-stranded RNAs (dsRNAs) that target gene promoters to induce transcriptional gene activation in a process known as RNA activation (RNAa). In this study we are going to investigate the likelihood to enhance the BMP2 expression in human Mesenchymal Stem Cells (hMSCs) using specifically designed saRNAs. If so, we can use the designed saRNAs as affordable small osteogenic factors in synthetic bone grafts and substitute materials instead of expensive BMP2 proteins with side effects.

Materials and Methods: First, Two different dsRNAs targeting BMP2 promoter are designed using short hairpin RNA target design tools including DSIR and Dharmacon. Secondly, the dsRNA inserts are separately ligated into pCDH vector, and then transformed using heat shock in home-made competent cells. In the following, target cells will be individually transfected with two kinds of constructs expressing BMP2 promoter-targeted saRNAs with the backbone construct serving as negative control. Using qRT-PCR, the relative expression of BMP2 and other marker genes in osteogenesis are measured at the mRNA level in both treated and control cells.

Results: Using target design tools we chose “GAATATATTATAGAAATATA” and “CTGCATTGTCCTGGATTTCG” sequences as the potent saRNAs to activate BMP2 gene expression. The sequences were successfully cloned. Following target cell transfection, The result would show whether our designed osteogenic dsRNAs could affect osteogenic pathway through targeting BMP2 promoter. Based on literature survey and saRNA target design tools, We believe our designed saRNAs hold a high potential to induce osteogenesis. Such a finding may have a great impact on gene therapy of bone associated disease, which would obviate the need for cloning of the large BMP gene by using a short RNA sequence instead.

Conclusion: Our finding will provide a novel approach to up-regulate the desired genes in different cells. However, the cell-type dependency of RNAa makes it tough to make a com-

mon algorithm for designing saRNAs. In addition, promoters consisting of certain elements such as the TATA box and CGIs (CpG islands) appeared to be more potent to be affected by dsRNAs.

Keywords: saRNA, RNAa, BMP2

Ps-67: Fabrication of Complementary Oxygen-Generating and Angiogenic Dual System for Heart Tissue Engineering

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Background: Acute myocardial infarction (AMI) ranks among the major causes of morbidity and mortality worldwide. Except for heart transplantation which is limited by donation deficiency and rejection, conventional therapies only treat the symptoms and cannot restore the damaged myocardium. Stem cell-based therapies represent a possible paradigm shift for cardiac repair; however, it faces several challenges. Ischemic myocardium has a harsh microenvironment for cell transplantation. Loss of blood vessels, hypoxia, acidic pH and high concentration of reactive oxygen species (ROSs) causes transplanted cell clearance within a month

Materials and Methods: To enhance cell survival and proper cell homing, we used two complementary methods simultaneously. First, by utilizing bifunctional core and shell-structured microparticles with Hydrogen peroxide as core and Poly-lactic-co-glycolic acid polymer (PLGA) as shell, short-termed oxygenation was occurred for about two weeks. Immobilized catalase enzyme on surface of microparticles degrades hydrogen peroxide, producing oxygen and also demolishing presented reactive oxygen species in the microenvironment which is toxic for cells. Second, for long-term oxygenation, we induced angiogenesis by employing fibrin-conjugated heparin injectable hydrogel for sustained delivery of angiogenic Vascular endothelial growth factor (VEGF). This arrangement is utmost goal because in one hand angiogenesis is occurred and on the other hand during the time-consuming angiogenesis process, cells are stayed away from hypoxic effects.

Results: After co-transplantation of microparticles and cardiac progenitor cells within an angiogenic injectable hydrogel to the left ventricle of rat AMI model, we expected significantly promoted graft function as evidenced by histology and echocardiography.

Conclusion: This study shows that exertion of oxygen generating-microparticles and induction of angiogenesis dual system is a promising method for Increasing the survival time of transplanted cells and improvement of damaged tissue.

Keywords: Acute Myocardial Infarction(AMI), Cardiac Progenitor Cell Transplantation, Oxygen-Generating Microparticles, Angiogenesis, Injectable Hydrogel

Ps-68: Time-Course Analysis of Direct Reprogramming of Cardiac Cells from Fibroblast

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Background: Mammalian heart has limited reprogramming potential to repair after embryonic development. Also, adult cardiomyocytes fail to recover during injury and disease. Hence, reprogramming of cardiac cell from different kind of stem and non-stem cells has a great potential for basic studies, pharmaceutical testes and therapeutic application and can be carry out as innovative strategies for replacing cardiac cells. Different strategies based on induction with transcription factor, microRNA or chemical cocktails have been developed that were induced in different time-point during early stage of reprogramming, from a few days to some weeks. Albeit, regulatory elements like transcription factor and their involvement pathways are not well define. In this study, we have used transcriptome datasets from three, seven and eighteen days during direct conversion of mouse fibroblast into cardiomyocyte to identify the key transcription factors and correlated signaling pathways.

Materials and Methods: In this study, differentially expressed genes with fold change equal or above 2 were isolated from total gene expression, using R language program. Then, differentially expressed transcription factors extracted by Enrichr online tools and in-house scripts. Also, Network analysis done using Cytoscape plugins to finding hub transcription factors. Finally, signaling pathways were obtained from KEGG and GO.

Results: Network Analysis have showed the most important differentially expressed transcription factors in three, seven and eighteen days during early stage of maturation of induced cardiomyocyte. Besides, we have introduced key signaling pathways during this conversion, based on their time point of reprogramming.

Conclusion: Our finding could be helpful to achieve regulatory elements involved in maturation pathway due to their time of induction. Also, would be useful in generating of more efficient induced cardiomyocytes for therapeutic applications.

Keywords: Direct Conversion, Network Analysis, Regulatory Factors, Signaling Pathway, Transcriptome

Ps-69: The Pore Size Effect of ECM-Modified Alginate for Human Embryonic Stem Cell-Derived RPE Attachment

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Background: Alginates are biocompatibility and biodegradability polysaccharide which can form hydrogel in the presence of divalent cations such as Ca²⁺. Without any modification, alginate is not suitable for cell attachment. Also, the porosity plays an important role. Porosity of alginate, should be suitable for cell growth, attachment and migration. Also it's important to infiltration of oxygen and the movement of nutrients and metabolic waste and prevent cell lack from the scaffold.

Materials and Methods: In this study, a thin layer of alginate was prepared by three different concentrations, 0.6%, 1.5% and 3% (w/v). Next, sodium alginate layer was gelled by adding a CaCl₂ solution. After that, the ECM was added to the alginate and dried at room temperature. Finally, the human stem cell-derived RPE were cultured on ECM-Alginate and the cell morphology was assessed.

Results: 10 days later, the cell morphology and expansion improved most effectively within 1.5% alginate gel compared with 0.6% and 3% alginates.

Conclusion: In this study, we designed alginate layer mimicking Bruch's membrane for hESC-RPE. Our results showed that alginate porosity could affect the attachment and expansion of RPE. Internal pore size of alginate gel increased with decreasing alginate concentration and increase in alginate gel porosity, that augmented the secretion of proteins and solutes from RPE cells, enhanced greatly cell attachment and maintenance. Therefore, the alginate layer with define pore size could be effective for RPE attachment.

Keywords: RPE, Alginate, Pore Size

Ps-70: Effect of Low-Level Laser Irradiation on Proliferation of Dermal Papilla Stem Cells

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Background: Dermal papilla (DP) is multipotent stem cell that plays important role in the hair follicle morphogenesis and regeneration. DP specialized mesenchymal component of the hair follicle that can be inducing hair follicle formation. It's noted that dermal papilla cell can provide a new approach to the cell therapy. Low level laser light can increase cell proliferation and also cause light reactions that referred to as photobiostimulation. So study of the low level laser irradiation effects on dermal papilla stem cells was done.

Materials and Methods: In this study, the effect of low level laser light on dermal papilla stem cell was investigated. The first, dermal papilla was isolated from the hair follicle. Characterization of dermal papilla was performed by flowcytometry. After laser power meter, the effect of level laser was investigated with the energy density of 5 and 10 J/cm² as a treatment.

Results: The result of this study showed that the low level laser light irradiation can be improved the proliferation of the dermal papilla stem cells.

Conclusion: Result of this investigation paves the way for utilizing the dermal papilla for fruitful applications in cell therapy and other its applications such as wound healing.

Keywords: Dermal Papilla, Stem Cells, Low Laser Irradiation

Ps-71: Novel Panel Expression of Long Non-Coding RNA as Reliable Biomarkers to Diagnosis Cholangiocarcinoma

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Background: The incidence of patients with Cholangiocarcinoma (CCA) as most prevalent primary biliary and hepatic malignancy has increased in the recent years. CCA was known as aggressive cancer because its survival time is less than 2 years. The surgery or liver transplantation is the only effective therapy for early-stage patients but unfortunately, most of them diagnosed at the advanced stages. The sensitivity and specificity of serum markers that frequently used in clinical such as carbohydrate antigen 19-9 (CA19-9) and CA-125 have lack of sensitivity and make mistake in treatment trend. In this study, we considerate Long non-coding RNA (lncRNA) as a member of non-coding RNA which exists in the nucleus and cytoplasm that have a length more than 200 nucleotides, as a novel biomarker for early detection of CCA. Today's many studies showed that lncRNAs have important roles in some functions such as regulating the post-transcriptional processing, splicing, transport, translation, and degradation processes of mRNA. Moreover, lncRNAs are closely associated with proliferation, migration, invasion, and tumor development, as a high expression of lncRNAs in some carcinoma promotes cancer to advance stages. Therefore, lncRNAs' different expression between tumoral tissues and normal ones can be used as a biomarker for early detection of cholangiocarcinoma.

Materials and Methods: In this systematic review study, an extensive English-language literature search was conducted using NCBI, ScienceDirect to identify original studies and review articles, according to keywords: Cholangiocarcinoma, Biomarkers and Long non-coding RNA till January 2018. The papers collected and then was ranking based on appropriate criteria. A total of 31 eligible studies were selected according to the keywords mentioned in the molecular field of cancer-related to Cholangiocarcinoma.

Results: After completing the final analysis, 26 studies from other research that were more relevant to this subject, were assessed and diagnostic accuracy lncRNAs were pooled using the "lncRNAs Disease databases".

Conclusion: According to the limited number of studies regarding the role of lncRNA in CCA, more studies will be needed in the future to demonstrate the importance of lncRNA in tumor initiation, invasion and metastasis, as well as their utility as therapeutic targets. Moreover, considering that lncRNA has been detected in pathological pancreatic tissues till now, the investigation in "easy access" samples like blood for the future will make them even more attractive and less invasive biomarkers.

Keywords: Cholangiocarcinoma, Biomarkers, Long Non-Coding RNA

Ps-72: Comparative Proteomics Analysis of Mouse Embryonic Stem Cell under Different Culture Conditions

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Background: Self-renewal and pluripotency maintenance of embryonic stem cell (ESC) could be controlled by the impact of culture condition in cell molecular mechanism. Understanding this mechanism is important for translating stem cell technologies to clinical applications. In this study, we aim to evaluate the proteome of mouse ESC-cultured under ground state conditions contain 2i and R2i (Royan 2 inhibitors of MEK and TGF β signaling pathways) in comparison with serum.

Materials and Methods: By the shotgun proteomics approach, we investigated the proteome of cells grown under 2i, R2i, and serum culture conditions. According to differentially expressed proteins, we choice loss of function approach to evaluating the related signaling pathway. The findings were validated by cell cycle analysis and gene expressions of the cells with flow cytometry and qRT-PCR, respectively.

Results: Out of 1749 proteins identified, ANOVA analysis revealed 171 differentially expressed proteins ($P < 0.05$) in 2i, R2i and serum. Among them, 120 proteins were significantly up-regulated and 51 proteins were significantly down-regulated between 2i and R2i versus serum. Gene ontology (GO) analysis of differentially abundant proteins showed that highest enrichment pathways in 2i- and R2i-cultured cells were associated with the metabolic process, glycolysis, gluconeogenesis and amino acid biosynthesis. Glycolysis was highlighted for energy production and used to maintain high levels of glycolytic intermediates to support cell proliferation which correlated with rapid cell cycling in 2i and R2i-grown cells. Focal adhesion, integrin signaling, and RNA transportation signaling pathways significantly down-regulated under ground state conditions. In terms of focal adhesion signaling, we confirmed the shotgun proteomics data for the integrins family by qRT-PCR, which showed reduced expression of the integrin subunits in 2i and R2i conditions. Integrins activation by Mn²⁺ in 2i and R2i cultures resulted in reduced Nanog level and increased the expression of lineage marker genes. The serum culture had more prominent phosphorylation of focal adhesion kinase (FAK) compared to 2i and R2i cultures which were related to increasing of focal adhesion signaling pathway. Therefore, reduced focal adhesion enabled mESCs to be maintained in an undifferentiated state.

Conclusion: Our results provided an insight into the key protein pathways, which are involved in self-renewal, and pluripotency maintenance employed by ESCs in ground state conditions different from that of serum culture.

Keywords: Mouse Embryonic Stem Cells, Proteomics, Energy Metabolism, Focal Adhesion, R2i

Ps-73: Multi-lineage Differentiation Potential of CD146+ Dental Pulp Derived Stem Cells

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Background: Routinely, cultures of dental pulp stem cells (DPSCs) are achieved by direct culturing of tissue derived single cell suspensions which are very heterogeneous and contaminated by fibroblasts. Recently we have shown that CD146 positive selection can discriminate between dental pulp fibroblasts (DPFs) and DPSCs. Here, we aimed to assess tri-lineage differentiation potential of CD146+ DPSCs.

Materials and Methods: Based on the CD146 expression, magnetic cell sorting was done to purify DPSCs from cultures of pulp derived cells. For adipogenic differentiation was done by culturing the cells in MesenCult medium supplemented with 10% Adipogenic Stimulatory Supplements (both from Stem Cell Technologies). Differentiation was assessed using oil red staining and PCR. For osteogenic differentiation, the cells were cultured in NH-osteodiff Medium (Miltenyi Biotec) according to the manufacturer's guidelines. To approve the differentiation, the cells were stained with Alizarin red and underwent RT-PCR. For neural differentiation, the media was replaced by neurogenic media (neurobasal medium, 2% B27, 20 ng/ml bFGF, 10 μ M retinoic acid) at passage 3-4. The media was changed every 2 days till 14 days. Differentiation was confirmed by Immunofluorescent staining for β -tubulin III. For hepatocyte differentiation, cells were serum deprived for 2 days and then were cultured in DMEM supplemented with 10 ng/mL basic fibroblast growth factor (bFGF) and 20 ng/mL epidermal growth factor (EGF). Then a two-step differentiation protocol was performed as follows: Step-1 was accomplished by adding DMEM supplemented with 10 ng/mL bFGF, 4.9 mmol/L nicotinamide and 20 ng/mL hepatocyte growth factor (HGF) for 7 days. In step 2, DMEM supplemented with 1 μ mol/L dexamethasone 20 ng/mL oncostatin M (OSM), 1.25 mg/mL bovine serum albumin (BSA), 10 μ L/mL ITS (insulin, transferrin, selenious acid), and 190 μ mol/L linoleic acid was added to the cells in order to complete cell maturation up to day 21 and hepatic differentiation was assessed morphologically and using RT-PCR.

Results: After induction of differentiation cells could change into appropriate morphological appearance and staining and PCRs confirmed successful differentiation into desired fates.

Conclusion: Our results reveal that the CD146 positive portions of the dental pulp derived cells were able to differentiate into all tri-lineages (mesoderm, ectoderm and endoderm) and are multipotent and to some-how pluripotent.

Keywords: Pluripotency, Dental Pulp Stem Cell, CD146

Ps-74: Resetting The Pancreatic Adenocarcinoma(PDAC) Cells into Non Tumorigenic Cells via Epigenetic Reprogramming Technology

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Background: Although cancer is generally believed to develop through accumulation of multiple genetic mutations, there is increasing evidence that cancer cells also acquire epigenetic abnormalities during development, maintenance, and progression. By utilizing the reprogramming technology as a tool to introduce the 'pressure' to alter epigenetic regulations, we might be able to clarify the epigenetic behavior that is unique to cancer cells. We hypothesized that using cell reprogramming technology would allow the Pancreatic adenocarcinoma cells to exit from tumorigenicity.

Materials and Methods: We therefore sought to reprogram PDX(patient derived xenograft) from human PDAC, by introducing (1) lentiviral mediated induction of Yamanaka Factors (OSKM), (2) the pluripotency associated gene OCT4 and the microRNA mir-302 and (3) Episomal vectors (OCT4, SOX2, KLF4, LMYC and LIN28A combined with P53 knock-down (shP53)) as a safe method to reprogram cells without genome integration. We compare these three different methods to find most efficient and safe method to reprogram PDAC PDX cells into less or non-tumorigenic cells.

Results: Immunostaining, Alkaline phosphates staining and real time PCR showed that induction with the episomal vectors is the most efficient method to reprogram our fibroblast and PDAC PDX cells and reprogramming of PDAC-PDX cells significantly altered their tumorigenic potential *in vitro* via differentiation-promoting effect of epigenetic reprogramming. *In vivo* results in nude mice clearly showed that direct reprogramming decreases the aggressiveness of PDAC-PDX 247 cancer cells as compared with its parental counterpart.

Conclusion: This study demonstrated that the Reprogrammed PDAC PDX cancer cells were distinct from natural PDAC cells with regard to their loss of tumorigenicity *in vitro* and *in vivo*.

Keywords: Epigenetic Reprogramming, Pancreatic Adenocarcinoma, Episomal Vectors

Ps-75: A Systems Biology Approach to Identify Signaling Pathway of Induction of Pluripotency by oct3/4, sox2, c-myc and klf4

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Background: Stem cells are the cells that have the capacity of self-renewing and maintain undifferentiated or differentiate to one or more types of cells. In these years novel researches on stem cell biology and technology have grown more. But the world of the cells is more complicated and each cell is interacting with multiple factors in the whole body. So it's very important to study this complex system together. The Study of biological systems by holistic approach is called systems biology. In 2008, Takahashi and Yamanaka designed a study for the induction of pluripotency in somatic cells. They discovered 4

factors for this reason: oct3/4, sox2, c-myc and klf4. But what is the importance of these factors? Are these factors critical in the induction of pluripotency signaling? Is it possible to substitute these factors to other factors? These are important questions that we should know for better work on induced pluripotent stem cell (iPSC). The best solution for knowing the answer to these questions is systems biology.

Materials and Methods: In this research signaling pathways that were touched by 4 factors of oct3/4, sox2, c-myc and klf4 are extracted from Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg>) and Reactome (<http://www.reactome.org>) databases. After that critical and confluence points in signaling pathways of these factors were determined and neighbor factors and a number of similar pathways of these neighbor factors to the mentioned factors determined. Finally, interaction network of all of the factors were drawn by Cytoscape software.

Results: It seems that the induction of similar pathways by different factors can help us induce pluripotency. But further experimental researches should be designed for analyzing this results in laboratory.

Conclusion: Bioinformatics and Systems biology approach can help stem cell biology and technology for starting novel researches with better lookout. In other words, we can check our idea by bioinformatics and extend our research in the whole system and after that experimental research will start based on computational and systematic results. So costs will decrease and results will be mindfully.

Keywords: Systems Biology, Stem Cell, iPSC, Cytoscape

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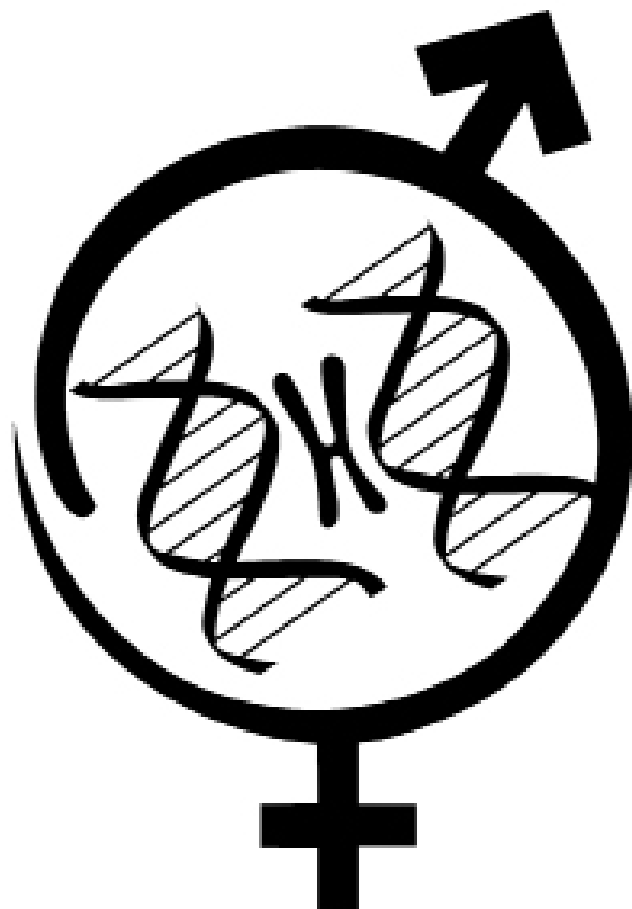
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Abstracts of
Royan International Twin Congress

19th Congress on Reproductive Biomedicine
29-31 August 2018

13th Seminar on Nursing and Midwifery
29-31 August 2018



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**Abstracts of the
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Congress Chairperson



Marziyeh Shiva

Dear Colleagues,

On behalf of the Organizing Committee, I would like to invite you to attend in the “19th Royan Reproductive Biomedicine Congress” (RRBC) that will take place on Aug 29-31, 2018 in Tehran, Iran. We have devised Scientific Programs with International communities through convention of Annual Royan International Research Award and twin Congresses on Reproductive Biomedicine (19th) as well as Stem Cell Biology and Technology (14th).

The scientific program is scheduled to the keynote speakers, plenary sessions, poster presentations and other programs such as educational and viable workshops.

We will try to cover all disciplines of ART protocols and its challenges from fundamental research to “precision medicine” applications in reproduction.

On behalf of the scientific committee of RRBC, it is my sincere pleasure to invite the distinguished and senior researchers to attend as invited speakers, the principle investigators and postgraduate students to participate actively by sending abstracts of their research results article or as audience since organizing committee intends to provide a wonderful forum for you to meet, interact and exchange your ideas with the Iranian and International outstanding scientists in this area.

We request you actively engaged in the conference and keep us proactive as well as support us shape the future of Reproductive Biomedicine. The members of the local organizing committee are very proud to be your host and look forward to welcoming you to our city and country.

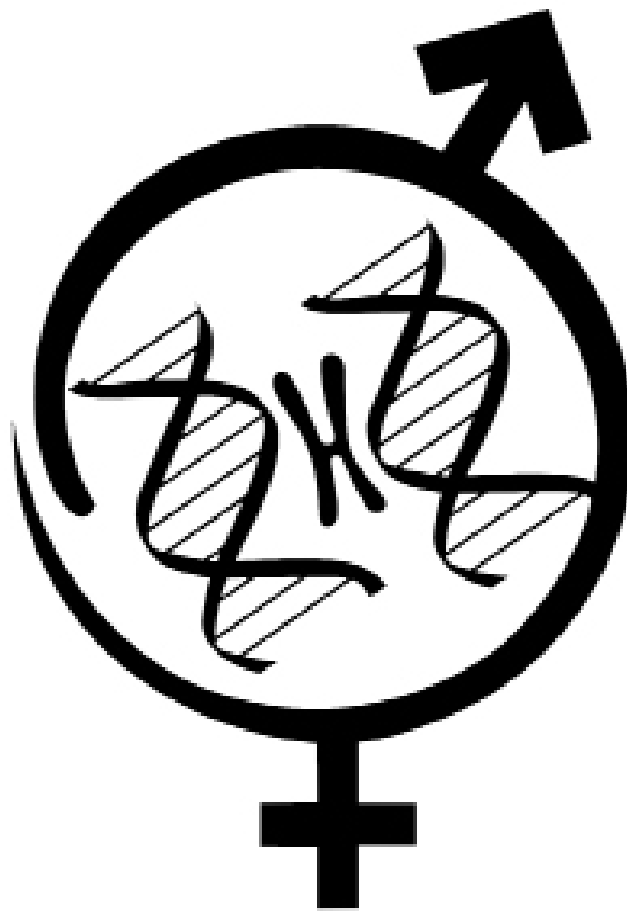
Marziyeh Shiva, M.D.

Congress Chairperson

19th Royan Congress on Reproductive Biomedicine

Abstracts of
Royan International Twin Congress

19th Congress on Reproductive Biomedicine
29-31 August 2018



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: 8-OHdG Detection in Human Sperm: A New Assay to Evaluate Sperm DNA Integrity

Drevet JV

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Study question: Can a discriminate threshold be determined for human sperm DNA oxidation?

Summary answer: A discriminant threshold was found with 65.8% of 8-OHdG-positive sperm cells and a mean intensity of fluorescence (MIF) of 552 arbitrary units.

What is known already: Oxidative stress is known to interfere with sperm quality and fertilizing capacity. However, current practice does not include the routine determination of oxidative DNA damage in spermatozoa; optimized consensus protocols are lacking and no thresholds of normality have been established.

Study design, size, and duration: Intra- and inter-methods comparisons between 4 methods were conducted to determine the most relevant and efficient means of assessing human sperm 8-OHdG content. Assay repeatability, specificity, sensitivity and stability were performed to validate an optimized methodology for routine diagnostic use.

Participants/materials, setting, and methods: This prospective study compared three immuno-detection methods including immunocytochemistry, fluorescent microscopy and flow cytometry. Sperm DNA oxidation for 80 patients was determined relative to semen parameters and clinical conditions, using the selected immuno-detection protocol in comparison with a commercial kit.

Main results and the role of chance: Significant positive correlations were determined for 8-OHdG values and sperm parameters using protocol III. MIF was notably highly and positively correlated with BMI and leukocyte concentration. Protocol III was the most discriminating method regarding assay repeatability, specificity, sensitivity, stability and reliability for sperm parameter alterations, in particular leukocytospermia. Of interest is that 39% of the subjects with “pathological” sperm DNA oxidation values were normozoospermic highlighting that DNA 8-OHdG evaluation allows the detection of conditions that could potentially help in the diagnosis of male infertility.

I-2: Sperm Nuclear DNA Oxidation: What to Be Afraid of? From Lab Models to the Clinic

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Normal embryo development as well as the health of the progeny are partly dependent on gamete nuclear integrity. If sperm DNA fragmentation that could be partly due to oxidative nuclear alterations is known to impact reproductive success, no one ever considers the impact of mild sperm DNA oxidative

damage. To analyze this situation, we have developed mouse models that display some level of post-testicular sperm DNA oxidative damage. The data presented will first focus on why mammalian spermatozoa are susceptible to DNA oxidation, and where these oxidative alterations are located in the mouse and in the human sperm nucleus and chromosomes. In addition, preliminary data will show that beside base oxidative alterations the sperm epigenetic information (with a particular focus on sperm DNA methylation status and the small non-coding RNA sperm pool) may be affected by post-testicular oxidative stress.

I-3: Diagnosis of Oxidative Stress / Sperm DNA Damage: What Clinicians Should Know Prior to ART

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A chronic state of oxidative Stress (OS) impacting the male reproductive tract is believed to be the primary cause of poor semen parameters and compromised sperm DNA integrity. In turn, poor sperm DNA integrity or sperm DNA damage is strongly associated with poor conception rates, increased incidence of miscarriage and the health of the next generation. As a result, the development of diagnostic assays for the direct or indirect detection of OS has gained much attention over the last two decades. Despite the commercialization of several such assays, only a few percent of ART couples are evaluated for OS or sperm DNA damage in the USA. The main reasons are; 1. many clinicians do not believe that sperm DNA damage is significantly consequential and therefore view such tests as “expensive add-ons”, 2. many clinicians require unequivocal clinical validation of the tests to predict pregnancy, 3. lab to lab testing variability and lack of reproducibility, 4. uncertainty over which test to use and finally and 5. unclear treatment protocol for men diagnosed with moderate to severe sperm DNA damage.

Following advancements in the field, the recent ESHRE policy guidelines now recommends testing for sperm DNA fragmentation, albeit limited to patients presenting with recurrent pregnancy loss. This short presentation aims to highlight the importance of assessing sperm DNA integrity as a matter of routine for all patients prior to treatment by ART.

I-4: Antioxidants in The Practice of Male Infertility; What Clinician Should Know Prior to Treatment by ART

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Oxidative Stress (OS) is now widely recognized as a critical male infertility factor affecting semen parameters, sperm DNA integrity, pregnancy and live-birth rates but most importantly the health of the offspring. Since OS is a manifestation of an insufficiency of cellular antioxidants, it stands to reason that clinicians should consider antioxidant therapy to ameliorate its deleterious effects. The question now is no longer if clinicians should adopt antioxidant therapy as part of their best practice, but which combination of antioxidants offer OPTIMUM efficacy and safety for their patients. This is not an easy task for healthcare practitioners to navigate, considering the worldwide availability of some 100 antioxidant formulations, each with

different compositions and ingredient doses. On the other hand, without any recommendation from the treating physician, men desperate to boost their fertility will choose antioxidant formulations at random. Many such arbitrary formulations, without published design rationale or credible clinical evidence, carry the serious risk of over-supplementation, which in fact may reduce fertility potential rather than improve it. Therefore, the need for convincing evidence from large quality clinical trials with antioxidants is now greater than ever. In the meantime, clinicians should still consider antioxidant therapy but pay meticulous attention to the evidence published for their design, safety and efficacy. In this short overview, we highlight the importance of antioxidant therapy together with guidelines aimed to provide greater awareness for clinicians to navigate this complex field.

I-5: Alcohol, Tobacco, Recreational Drugs and Steroids: Alterations in Testicular Function and Semen Quality

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Anabolic Steroids: Since its isolation and characterization in 1935, testosterone has been further studied, leading to the synthesis of numerous derivatives with different properties from the original molecule. These derivatives are called “anabolic-androgenic steroids” (AAS), or more commonly, “anabolic steroids”. Initially, these substances were restricted to professional athletes and bodybuilders, becoming gradually more popular among recreational and non-professional power athletes. Current estimates indicate as many as over 50 million AAS users in the world. Interestingly, two thirds are noncompetitive bodybuilders, or even non-athletes, using these substances with aesthetic or performance purposes only. In addition to it, steroids may also be found in “dietary supplements”, which were supposed to be AAS-free. International surveys recently reported an overall steroid contamination rate of 15-25%, depending on the country. AAS abuse is, therefore, an issue of major public health concern, considering its increasing prevalence. Infertility is defined as the inability to achieve natural pregnancy after 12 months of regular unprotected intercourse, with the male partner being solely responsible or in combination with the female in 50% of all infertile couples. Environmental and lifestyle issues are of increasing concern and specially related to male infertility, besides the traditional causes like varicocele, obstructive and non-obstructive azoospermia, infection, genetics, etc. Some reversible some not, being hypogonadotropic hypogonadism a typical example of a known reversible condition, while primary testicular impairment is often related to a less reversible one. Structural modifications have been introduced into the testosterone molecule in an attempt to maximize the anabolic effect and minimize the androgenic ones. However, all AASs are virializing if administered for long enough, at high enough dosages. Therefore, they are synthetic derivatives of testosterone, and not only testosterone itself. The AAS structural base is the “steran nucleus”, consisted of three condensed cyclohexan rings in nonlinear junction, and a cyclopentane ring. The anabolic effects are dose dependent, and usually occur when supra physiological testosterone levels (higher than 1000ng/dL) are found, which generally requires weekly doses of 300mg or more. Besides, AAS may also be classified con-

sidering their main activity: 1. “Testosterone-like” effect, very potent, with great muscle strength gains. They usually show an anabolic/androgenic rate close to 1:1, similar to testosterone itself. The high aromatization rates are also comparable to testosterone’s. They include all testosterone esters, methyltestosterone and others. 2. “Dihydrotestosterone-like” (DHT-like) effect, potent but highly androgenic. Since they resemble a 5DHT molecule, they basically cannot be aromatized to estrogens. It also explains the low water and salt retention of drugs of this group. They include stanozolol and oxandrolone. 3. “Nandrolone-like” effect, the less potent of all, with the highest anabolic/androgenic rate. They have some progesterone-like activity, inhibiting the hypothalamic axis. These are the mostly used drugs in the clinical setting, when anabolic effects are desired (reversing catabolic states, such as AIDS associated cachexia, severe burns, and chronic obstructive pulmonary disease). They include the nandrolone esters and trembolone.

Crack-cocaine: Cocaine consumption and addiction has become a global, unresolved, and increasingly legal and health issue in Western countries, reaching alarming epidemic proportions (Withers et al., 1995). Within its borders, Brazil has experienced increased cocaine consumption in an affordable and smokable freebase form that is six-fold more powerful and more addictive than the cocaine powder, i.e. “crack-cocaine”. The crack-cocaine processing starts by pressing the leaves of the *Erythroxylum coca* together with sulfuric acid (H₂SO₄) to produce the cocaine powder. The crack-cocaine is produced by dissolving powdered cocaine in a mixture of water and sodium bicarbonate (NaHCO₃, baking soda), in a 1:1 ratio to reduce the costs of cocaine production. The mixture is boiled until a solid substance form. The solid is removed from the liquid, dried, and then broken into the chunks (rocks) that are illegally sold as crack. In 2010, the government of Brazil launched an integrated plan to combat the trafficking and consumption of crack-cocaine and other drugs (INCB, 2010). The use of crack-cocaine is widespread in large cities, and crack-cocaine has become popular among young adults, particularly teenagers, because of the “advantage”. Demand for crack-cocaine appears to be emerging in South American countries; in 2008, seizures of crack-cocaine were reported in Argentina, Brazil, Chile, Paraguay and Venezuela (INCB, 2010). The human testis is a known target organ for injury resulting from exposure to pharmaceutical and environmental agents. Today there are over 7 million crack addicts in Brazil alone, being the largest number of addicted in the world. This is a public health crisis of enormous proportions. Risks for other countries are tremendous and actions should be fearless taken to avoid its dissemination particularly in adolescents and young adults.

The known central and peripheral pharmacological effects and systemic intoxication by intravenously injected cocaine may have gender-related differences, with more deleterious effects in male mammals (in which lower levels are sufficient to cause testicular toxicity. Additionally, there are deleterious effects on testicular function in rats of different ages, which is serious for peripubertal male rats.

Marijuana: Marijuana, the popular name for dried *Cannabis sativa*, is the most consumed drug worldwide. The main compound and most potent psychoactive agent, delta-9-tetrahydrocannabinol (THC), acts as a negative competitor to the endogenous cannabinoid anandamide, by interacting with specific receptors. These receptors are expressed mostly in the central nervous system (CNR1) and sperm (CNR1 and CNR2) affecting sperm motility and acrosome reaction. In particular for the marijuana study.

Background: To examine the effects of Marijuana consumption on seminal parameters and hormonal levels in men.

Materials and Methods: Individuals were evaluated with physical examination (testicular volume) using an orchidometer, a pachymeter, Doppler-stethoscope, ultrasound by Doppler-color, complete semen analysis. Seminal analysis was performed according to the WHO's guidelines and morphology also according to the Kruger's criteria biochemical markers of sperm function and functional tests, including: creatine-kinase (CK), anti-sperm antibodies, reactive oxygen species (ROS), DNA fragmentation (SCSA). The study included marijuana users (study group) aged 21 to 58 years-old, and pre-vasectomy patients (control group) from both the private laboratory Androscience and university-based andrology evaluation for the controls. Data analysis was performed using the Wilcoxon test and a linear model of gamma distribution to extract the age effects on each parameter; p value of <0.05 was adopted.

Results: Significant effect of Marijuana was observed in hormone levels, seminal pH, total sperm count, total progressive motility and both WHO and Kruger morphology ($P < 0.001$).

Conclusion: The consumption of *C. sativa* has a significant negative effect on male reproductive health, reflected in a significant disturbance in some hormone levels and a significant reduction in sperm quality.

Alcohol: Alcohol is a psychoactive substance that may create dependence and affects the overall health of man by different mechanisms, which can be fatal in excessive chronic use. The possible association between alcohol consumption and reduced male fertility has been the subject of several studies and still remains unclear.

Background: To demonstrate the effects of alcohol consumption in fertile men of reproductive age, evaluating semen parameters and hormonal profile.

Materials and Methods: For this study, were included data of semen analysis, hormone profile and testicular volume of pre-vasectomy candidates with no risk factors for sperm/testicular dysfunction from University-based public hospital and from a private setting - Androscience, High complex Clinical and Research Andrology Laboratories. The study was approved by Ethics Committee (12331/14). We conducted T-Test for independent samples and adopted $P < 0.05$.

Results: Subjects who reported not being drinkers constituted the control group, mean age of the patients 34.7 ± 5.43 . Who declared themselves consumers was included in alcohol group, mean age 37.35 ± 6.55 . Statistical differences were seen in following seminal parameters: pH (7.65 vs. 7.97; $P < 0.07$), motility grade A (8.59 vs. 4.61%; $P < 0.003$), strict criteria (6.46 vs 4.11%; $P < 0.01$) and WHO (21.44 vs. 15.22%; $P < 0.004$) normal morphology and total number of round cells (14.75 vs. 5.66 million; $P < 0.001$). In hormonal parameters, there was an increase of 17-OH progesterone in alcohol consumptions (1.05 vs. 1:54ng/mL; $P < 0.011$), as well LH levels in wine consumption.

Conclusion: In view of results, we suggest that alcohol intake affects adversely the production of 17-OH progesterone and spermatogenesis, resulting in reduced motile and morphological quality of sperm. Thus, the intake of alcohol appears to be associated with reduced male reproductive potential and should be advised to be ingested in limited amounts.

Keywords: Anabolic Steroids, Cocaine, Marijuana Smoking, Tobacco use Disorder, Spermatozoa, Testis, Free Radicals, Oxidative Stress, DNA, Hypogonadism, Male Infertility, Sex Hormones, Testosterone

I-6: How Environmental and Air Pollution Disrupt Spermatogenesis and Male Reproductive and Sexual Health: An Epidemiological and Mechanistic Approach

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Background: Through a series of research projects and experiments, we have demonstrated the link between environmental and air pollution and general health of an individual and a population living in industrialized and urban city (Sao Paulo) using male infertility and erectile function/dysfunction, but not limited in their own scope of disease, but also in a broader perspective as bio markers of global reproductive and sexual health in a population-based analysis. Also, we analyzed in two studies if positive government mitigating policies redounded in improvement of the same parameters. Sex ratio at birth, or secondary sex ratio (SSR), can be understood as the ultimate result of the battle between maternal and paternal genomes. In this battle of gene interests, sex determination, genomic imprinting, X chromosome inactivation, and meiotic drive are the main mechanisms regulating sperm and oocyte production, fertilization, nearly development, fetal survival, and reproductive fitness. Sex ratio skew in which a female-biased litter may occur in some mammalian species under both field and experimental stressful conditions, such as food availability and air pollution, but have been mainly studied from the oocyte perspective, not from the male gamete perspective.

Materials and Methods: A series of epidemiological, observational and research projects gathering in each one from hundreds of individuals to hundreds of thousands to even millions of births involving sex-ratio disturbances in polluted areas to semen quality alterations in populations and/or workers heavily exposed to vehicular emissions and biomass burning where we obtained data from 205,000 births from selected cities with over 100 million tons/cane production per year and collected information on the extension of the sugar cane plantation area and number of foci of fires on a daily basis by satellite monitoring, and subdivided them in five categories according to the relationship between cane plantation area and the role area of the city: 0-15%, 16-30%, 31-45%, 46-60%, >60%. In another study, semen quality was used as end-point in pre-vasectomy fertile patients over almost a decade to analyze prospectively the effects of air pollution. Using erectile function as a bio marker, we conducted an epidemiological study in the Amazon rain forest to study how an isolated population with good health habits and non-industrialized food consumption in an environmentally-free of pollution stands for sexual quality of life. Reactive oxygen species and sperm quality tests were performed in some studies.

Results: I will point out results, study by study. Study on decreased semen quality in a fertile population: Data between 2000 and 2008 from 743 patients aged between 23 and 50 years (mean 36.5 years old) in the city of São Paulo clearly demonstrates a decrease in semen parameters in healthy donors. Seminal analysis was performed according to the WHO and strict criteria. Daily data of air concentrations of O_3 , CO, NO_2 , SO_2 , and PM10 were collected and plotted to the specific individuals exposed. Seminal analysis parameters (concentration, motility, morphology) were correlated with daily data of air pollution separated into quartiles and corresponding to collection date.

Sperm concentration (million/mL; $r=-0.06$, $P=0.079$) and morphology (%WHO; 0.014 , $P=0.699$) were not statistically different within different ages, but progressive motility (A + B%) showed negative correlation with time in these 8 years' time (%; $r=-0.18$, $P\leq 0.001$). Studies on sex-ratio: We have elegantly demonstrated a significant sex ratio decrease in the most polluted areas of São Paulo city, Brazil. Results disclosed a significant negative association ($r^2=0.7642$, $P=0.013$) between SSR and PM10 within a relatively narrow range of PM10 levels (31-61 $\mu\text{g}/\text{m}^3$). In the least polluted area the sex ratio was 51.7% (106.8) for 34,795 births recorded, and for the most polluted area the proportion decreased to 50.7% (102.9) for 48,023 births recorded. This result corresponds to a difference of 1% in total male births or 1.180 fewer male births in the highest polluted areas per year. Similar findings were observed in the experimental study. After exposure, reproductive mature and nonexposed virgin female mice were placed in the chambers to mate. Therefore, male Swiss mice housed 10 days after birth in open top chambers exposed to air pollution, where they mature and mate to nonexposed virgin female mice, produced an offspring with a 0.86 male/female ratio. The offspring of the group of mice concurrently raised in a similar but filtered open-top chamber were significantly ($P=0.042$) higher (1.34). Sex ratio was studied in 27 million births in five major regions of this continental country and compare results from the United Nations Index of Human Development. Interestingly, we found that sex ratio is decreased in the southeast and southern regions, which are by far the most developed, industrialized, and polluted areas. Results are: 1. North (51.41%), 2. Midwest (51.39%), 3. Northeast (51.37%), Southeast (51.16%), and South (51.22%). Statistical differences: Northeast versus Southeast ($P=0.002$); North versus Northeast ($P=0.0041$). In the sugar-cane biomass burning results demonstrated a decrease in sex ratio by 0.3% ($P=0.01$). Results demonstrated sex ratio reestablishment with decreased PM10 over 5 years in Sao Paulo in large areas where mitigation policies were introduced. In the study of traffic-controllers our results indicate a decrease in progressive motility much above the already decreased motility in the control group exposed to regular air pollution and both are worse when compared with pre-vasectomy patients of control group. nonpolluted areas. Morphology by strict criteria was also significantly reduced and antisperm antibodies were higher in the traffic controllers than in the control group. Finally, quality of sexual life is maintained in the Amazon isolated-area population-based study, even at older ages of 65 to 70 y.o. and can be compared with much younger individuals in the same region. The response rate was 81.69%. The mean age was 36.00 ± 12.95 years, and most men had mixed ethnicity (63.11%), were self-employed (42.07%), had a monthly earned income of US\$0 to US\$460 (46.75%), and were single (36.10%). The mean MSQ score was 80.39 ± 12.14 (highly satisfied). None of the demographic characteristics showed a statistically significant influence on sexual satisfaction. The difference in quality of sexual life was statistically significant compared with age ($P<0.01$). The domains of desire ($P<0.01$), partner satisfaction ($P<0.04$), and erection quality ($P<0.01$, $P<0.03$, $P<0.02$) were statistically significant.

Conclusion: There is compelling evidence of the harmful effects of exposures to environmental pollution on reproductive health from our studies. One conclusion that can be drawn is that the sex ratio skew presented here represents a primary sex ratio (PSR) skew instead of an SSR skew often caused by preferential males' losses, once the litter sizes were similar and no increase in abortions was seen. If the PSR is reduced, we must hypothesize that the histological differences presented by the

exposed animals can represent only the tip of the iceberg of other deeper testicular modifications. Molecular and chromosomal events such as altered imprinting patterns, X-Y pairing errors, and oxidative stress could have led to skew or skill "superiority" of X-bearing sperm. As a conclusion for this part of the chapter, sex ratio and its variants can be used as bioindicators of reproductive health and help scientists with powerful tools and arguments to delineate government policies. Preserved quality of sexual life in the Amazon was a demonstration that men's health is linked to the environment that surrounds each individual, we were not designed to so many stressful conditions imposed by post-modern society in the last hundreds of years.

Keywords: Environmental Air Pollution, Reproductive Health, Male Infertility, Erectile Dysfunction, Spermatozoa.

I-7: Microfluidics for Male Fertility

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Background: Infertility is a growing global health issue with far-reaching socioeconomic impacts, affecting >50 million couples worldwide. Semen analysis and sperm selection are cornerstones of infertility diagnosis and treatment. However, current clinical methods for sperm analysis and selection are insufficient, costly, time-consuming, and prone to operator error, resulting in sub-optimal pregnancy outcomes. The global trend of rising infertility motivates immediate attention to infertility issues, and highlights the fundamental challenges of making both diagnosis and treatment affordable and accessible. Here, rapid and low-cost microfluidic methods are demonstrated for the study, analysis, and selection of sperm for male infertility diagnosis and treatment.

Materials and Methods: Microfluidics provides several fruitful opportunities for infertility diagnosis and treatment. Microfluidic methods were applied here to (a) understand the biophysics of sperm motion, (b) develop paper-based strategies for semen analysis, and (c) develop rapid technologies for high-quality sperm selection. First, microfluidics and total internal reflection fluorescence microscopy were used to capture and describe full 3D dynamics of sperm motion near surfaces with nanoscale resolution for the first time. Second, by leveraging biochemical and electrokinetic capabilities in paper-based assays, affordable and accessible technologies were developed for at-home semen analysis and sperm DNA integrity testing. Finally, by leveraging the natural swimming characteristics of sperm to follow boundaries in a parallel array of 500 microchannels, a rapid sperm selection device was developed that reflects the natural *in vivo* process. These microfluidic devices were clinically tested with human samples against current best practices.

Results: A distinct 2D 'slither' swimming mode was discovered for sperm within $1 \mu\text{m}$ of a surface, in which the full sperm body is aligned parallel to the surface and the flagellum beats in a 2D plane. Human sperm swims 50% faster in the slither mode, suggesting a strategy that is well-suited to the confined portions of the reproductive tract. With respect to semen analysis, a rapid (10 min) and low-cost (US\$0.05/device) paper-based technology was developed for at-home male fertility testing that simultaneously quantifies three critical semen parameters (concentration, vitality, and motility) using a colorimetric enzymatic

assay. The paper-based device provided 100% agreement with conventional CASA and dye exclusion vitality assay in terms of clinical outcome for patients. Additionally, a rapid, sensitive, and low-cost paper-based approach was developed for sperm DNA integrity analysis that provides identical clinical outcome as flow cytometry-based SCSA, with two orders of magnitude less overall capital and operating costs. With respect to sperm selection, a high-throughput microfluidic technology was developed for one-step semen purification and high DNA integrity sperm selection, by on-chip processing of 1 ml of raw semen in <20 min. Clinical tests with raw human semen samples showed >80% improvement in DNA integrity of selected sperm, considerably outperforming the best practices in current use.

Conclusion: Microfluidics has led to new biological insights into sperm behavior, and developments in microfluidic devices show the most promise for near-term medical advances in male fertility. These technologies are scalable alternatives to conventional clinical testing and provide novel opportunities to alleviate major emotional and financial burdens for families dealing with infertility worldwide.

Keywords: Male Infertility, Microfluidics, Sperm, Semen Analysis, Assisted Reproduction

I-8: Andrological Surgery in ART

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I-9: Role of Sperm DFI on IVF/ICSI Success Rate

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Traditionally, evaluation of semen parameters has been used to differ fertile men from infertile ones. Today, the use of more accurate diagnostic methods with a more efficient tool has made careful evaluation of the structure, quality and integrity of sperm DNA for management of male infertility.

Regardless of the type and the origin of produced sperm DNA damage, it seems that damage to DNA structure and integrity has an effect on fertilization, embryo quality, pregnancy rate and miscarriage and abortion rate during the use of *in vitro* fertilization and intra-cytoplasmic sperm injection. Origin of produced sperm DNA damage may be due to internal pathways such as ROS production, or defects in chromatin condensation, or due to external pathways such as high risk occupations, chemicals and toxic component or life style and nutrients. The routine and widespread use of sperm DNA fragmentation evaluation in the infertility centers and laboratories is not yet supported. It seems, more studies are needed to standardize the measurement of sperm DNA damage and to determine the exact role of sperm DNA damage in a large number of other female and male factors leading to fertility results in IVF and ICSI.

In this session, the effect of sperm DNA damage in male infertility and IVF, ICSI outcome are described, and then some therapeutic approaches to reduce and improve sperm DNA damage are addressed.

Keyword: Sperm DNA Integrity, Assisted Reproduction Techniques

I-10: Iranian Temporal Changes in Semen Quality: 22 Years of Experience

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Although there are numerous reports about temporal changes in semen quality from all over the world, debates still continue. In this article we have compared the semen quality of 707 Iranian male patients between 1990 to 1992 (group 1) with 1108 men from 2010 to 2012 (group 2). We showed despite increased sperm concentration from 84.48 in group 1 to 95.55 in group 2, sperm with normal morphology decreased significantly from 62.2 to 44.44 %, grade A motility decreased significantly from 38.6 to 30.6%, grade B motility increased significantly from 21.34 to 30.3 % and grade C and D motile sperm remained constant. We also assessed the effect of age on semen parameters between 2 groups. These results should be further evaluated by larger sample groups in the future.

Animal Biotechnology

I-11: Genetic Engineering in Model Livestock Species

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Background: Genetically engineered farm animals are attractive alternative mammalian models to rodents for the study of developmental, genetic, reproductive and disease-related biological questions. However until recently, the molecular methodologies for genetic engineering in these species were not established. The development, refinement and optimization of molecular methods for precise genetic modifications are necessary to fully exploit the potential of these species for translational research.

Material, Methods and Results: DNA-Transposons, Recombinases and Crispr/Cas9 designer nucleases have been employed for direct injection in murine, porcine and bovine zygotes to achieve precise genetic modifications.

Gain-of-function and loss-of-function modifications have been achieved, and a simplified injection technique has been established. Importantly, the cytoplasmic plasmid injection (CPI) is also suitable for opaque zygotes from pigs or cows.

Conclusions: Although the transgenic toolbox for large animals is currently equipped with powerful methodologies there are many aspects to improve in the associated reproductive technologies. Low success rates of animal cloning (SCNT) and zygote microinjection, two of the most commonly used method, still represent a bottleneck. The ground-breaking feature of the Crispr/Cas9 system is that it brought the possibility of purposefully directing the genomic modification to a specific chromosomal locus. It is anticipated that new generation transgenic tools in concert with updated genomic data will facilitate the production

of large animal models for translational medicine. These large animal models will be instrumental for understanding disease pathogenesis and development of better therapeutic approaches of severe human pathologic conditions.

I-12: Non-Viral Derivation of Induced Pluripotent Stem Cells from Farm Animals

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Background: The recent establishment of induced pluripotent stems (iPS) cells promises the development of autologous cell therapies for degenerative diseases, without the ethical concerns associated with human embryonic stem (ES) cells. Initially, iPS cells were generated by retroviral transduction of somatic cells with core reprogramming genes. To avoid potential genotoxic effects associated with retroviral transfection, more recently, alternative non-viral gene transfer approaches were developed. Before a potential clinical application of iPS cell-derived therapies can be planned, it must be ensured that the reprogramming to pluripotency is not associated with genome mutagenesis or epigenetic aberrations. This may include direct effects of the reprogramming method or "off-target" effects associated with the reprogramming or the culture conditions. Thus, a rigorous safety testing of iPS or iPS-derived cells is imperative, including long-term studies in model animals. This will include not only rodents but also larger mammalian model species to allow for assessing long-term stability of the transplanted cells, functional integration into the host tissue, and freedom from undifferentiated iPS cells.

Material, Methods and Results: Here, we generated iPS cells from rodent and farm animal species by a non-viral approach. The iPS cells showed all characteristics of pluripotent stem cells, including morphology, alkaline phosphatase expression, and typical hallmarks of pluripotency, such as expression of pluripotency markers and formation of mature teratomas in immunodeficient mice.

Conclusions: These results are promising for derivation of germ line-competent iPSs and will facilitate to assess the safety on novel stem cell therapies in relevant large animal models.

I-13: The Natural Toxins Do not Discriminate between Male and Female

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An overwhelming number of evidence has accumulated during the last decade indicating a negative impact of environmental contaminants on reproductive health. Equally most of the experts do believe that prevention is an important issue in reproductive environmental health as toxic agents likely are able to pass on to future generations. To do so, it is essential to recognize these toxic materials and their impacts on reproductive systems in males and females. Mycotoxins as one of the ubiquitous natural contaminants will be discussed in this review paper. During the last two decades our research team focused on "effects of various mycotoxins on reproductive systems including on sperm quality parameters, *in vitro* fertilization rate,

hormonal situations, histopathological changes, cellular and molecular pathways in laboratory animals model and in both males and females". This piece of research was performed with variety of toxins such as zearalenone, deoxynivalenol, Aflatoxin B1, cyclopiazonic acid, and α -zearanol. Our broad range of findings spread from simple reproductive Sertoli cells cytotoxicity induced by deoxynivalenol to the reduction of leydig cells steroidogenesis and disruption of mitosis infrastructure by zearalenone and AFB1-induced overexpression of p21. Our findings suggest that these compounds do affect both genders and all pathways in pathophysiology of related disorders.

Keywords: Natural Contaminants, Mycotoxins, Reproductive System

I-14: Improved Tissue Cryopreservation Using Inductive Heating of Magnetic Nanoparticles

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Vitrification, a kinetic process of liquid solidification into glass, poses many potential benefits for tissue cryopreservation including indefinite storage, banking, and facilitation of tissue matching for transplantation. To date, however, successful re-warming of tissues vitrified in VS55, a cryoprotectant solution, can only be achieved by convective warming of small volumes on the order of 1 ml. Successful re-warming requires both uniform and fast rates to reduce thermal mechanical stress and cracks, and to prevent re-warming phase crystallization. We present a scalable nanowarming technology for 1- to 80-ml samples using radiofrequency-excited mesoporous silica-coated iron oxide nanoparticles in VS55. Advanced imaging including sweep imaging with Fourier transform and microcomputed tomography was used to verify loading and unloading of VS55 and nanoparticles and successful vitrification of porcine arteries. Nanowarming was then used to demonstrate uniform and rapid re-warming at $>130^{\circ}\text{C}/\text{min}$ in both physical (1 to 80 ml) and biological systems including human dermal fibroblast cells, porcine arteries and porcine aortic heart valve leaflet tissues (1 to 50 ml). Nanowarming yielded viability that matched control and/or exceeded gold standard convective warming in 1- to 50-ml systems, and improved viability compared to slow-warmed (crystallized) samples. Last, biomechanical testing displayed no significant biomechanical property changes in blood vessel length or elastic modulus after nanowarming compared to untreated fresh control porcine arteries. In aggregate, these results demonstrate new physical and biological evidence that Nanowarming can improve the outcome of vitrified cryogenic storage of tissues in larger sample volumes.

I-15: Feasibility Study in The Development of Whole Ovary Vitrification and Nanowarming

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Ovary, uterus, and testis banking could restore fertility and hormone balance to the 140,000 childhood and young adult cancer survivors in the U.S. each year and hundreds of thousands more worldwide. Early successes suggest that ovarian tissue

and even whole ovary banking is possible and could restore fertility and function. This feasibility study in a porcine model used a multi-faceted banking approach targeting human reproductive organ banking including ovary preconditioning, loading of the vitrification solution, M22, at subzero temperatures, and loading targeted quantities of iron oxide nanoparticles for subsequent rapid radio-frequency warming, "nanowarming". Whole porcine ovaries were procured and flushed with 10 ml ice-cold heparinized (1000 U/ml) Unisol cold storage solution. Preconditioned ovaries (n=7) were perfused at $4 \pm 4^\circ\text{C}$ with flow rates between 0.5 and 1.2 ml/min, followed by stepwise-loading of M22 at subzero temperatures (n=5) using a custom-built multi-thermic machine perfusion (MTMP) system. The MTMP system achieved a cooling rate of $-2^\circ\text{C}/\text{minute}$ from 0°C to -10°C and $0.2 -1^\circ\text{C}/\text{min}$ until reaching the coldest temperature, -16.6°C , during the final loading step. M22 equilibration was sufficient to achieve vitrification of ovaries (n=4) in a 10 ml volume when plunging in liquid nitrogen. Finally, iron oxide nanoparticles were loaded in a dose-dependent manner (n=17) with concentrations of 5-15 g/ml of iron. Particles were observed to be distributed within the vasculature of the the col-layer of antral follicles (by histology) and preliminary measurement of warming rates correlated with the infused nanoparticle concentration, achieving a 4X increase ($3.58^\circ\text{C}/\text{min}$) compared to convective warming ($0.85^\circ\text{C}/\text{min}$) from 0° to 22°C . These early results demonstrated the feasibility of a comprehensive protocol for whole ovary banking, including hypothermic preconditioning, vitrification, and subsequent nanowarming. Additional optimization of these comprehensive methods could make ovary banking clinically feasible and yield insights applicable to banking testicular tissue, whole testes, and larger organs.

I-16: Role of NANOG in Goat Pre-Implantation Embryonic Development

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Pluripotent cell-specific gene including Nanog plays essential roles in regulation of signaling pathways, especially in maintenance and induction of pluripotency in embryonic stem cells (ESC) and inner cell mass (ICM) in many species including mouse and human. Unlike in these species, the molecular features and transcription regulation of NANOG gene in goat is not well defined. In this study, by knockdown of NANOG mRNA in goat embryos, we examined the role of this gene on early embryonic development. Therefore, 8-10 pl of NANOG or scrambled (SCR) siRNA were injected into presumptive zygotes. Subsequently cleavage and blastocyst formation rates were assessed along with gene expression analysis in 6-8 cell and blastocyst stage. In siRNA groups, cleavage and blastocyst rates were slightly but insignificantly lower than the control and SCR groups, however, embryos with reduced expression of NANOG presented reduction in number of trophectoderm and total cells in blastocysts. Gene expression analysis of developmentally important genes, including: SOX2, OCT4 and NANOG, showed that this reduction results in significant increase in expression of SOX2 and OCT4 and amid the conceivable target genes (CDX2, REX1 and GATA4) of this network, only

GATA4 displayed increased expression. Therefore, we concluded that NANOG is likely to be essential for proliferation of trophoblastic cells.

I-17: What Did Animal Model Studies Give us about The Varicocele-Induced Pathogenesis both at Testicular and Sperm Levels: A Fact or Fiction

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The varicocele (VCL), as inevitable fact of infertility reason, has been reported in 10-20% of infertile individuals. The VCL is categorized into three different grades, initiation from faint to more severe conditions, which is able to potentially result in complete infertility. Indeed, diminished venous drainage, retrograde blood flow-down to pampiniform plexus, and increased temperature of testicles have been illustrated as prevailing reasons of VCL-induced pathogenesis in testicular tissue, leading to detrimental impact on spermatogenesis and spermiogenesis. Additional theories, which explain the testicular effects of VCL are suboptimal drainage of testicular gonadotoxins due to venous dilatation, reflux of renal and adrenal metabolites contributing to venous dilatation, testicular hypoxia, higher levels of oxidants in the semen, and anti-sperm antibodies. In line with this issue, several studies have shown that, the VCL adversely affects the germinal epithelium and sperm cells on a cellular and molecular level, including diminished testicular DNA polymerase activity, enhanced germ and sperm cells apoptosis, elevated reactive oxygen species (ROS, named as VCL-induced oxidative stress), altered Sertoli cell niche and network with Leydig cells, and finally decreased Leydig cell testosterone secretion. Among all these hypothesis, those trials performed using animal models established the VCL-induced pathogenesis mechanistically. Accordingly, it has been clear that, the VCL-induced testosterone withdrawal results in a cascade of evidences, leading to impaired chaperone-related testicular homeostasis, including DNA fragmentation, and protein degradation, and negatively affects the Sertoli cells-related microenvironment and/or niche, resulting in suppressed spermatogonial stem cells self-renewal process. Moreover, the animal model-based trials have illustrated that, the VCL-induced oxidative stress results in severe DNA damage at precursor germ cell level, and negatively affects the cell cycle machinery during mitosis and meiosis, resulting in spermatogenesis arrest. In line with these findings, using animal models, demonstrated that, the VCL-induced oxidative stress down-regulates the potential in stabilizing mRNA content of haploid germ cells, and via this mechanism it negatively affects the essential protein synthesis. Moreover, it came clear that, the progressive oxidative stress in association with overexpression/synthesis of inflammatory mediators (such as TNF- α , IL-1, IL-6, IL-10, iNOS, ALP, AST) in VCL-induced testicles negatively affects the testicular endocrine status, resulting in arrested/delayed spermatogenesis and spermiogenesis. In addition, the animal model studies showed the cross-link between oxidative stress, hypoxia, and suppressed endocrine status with epigenetic alterations during histone-protamine replacement process. Finally, using animal model studies made it clear that, the long-time VCL is able to result in pre-implantation embryo arrest at very early stages, and diminishes the blastocyst ratio. Thus, considering all these findings, it would be more logic to conclude that, the animal model trials could successfully help the researchers and clinicians to understand the molecular, bio-

chemical and histological changes during VCL in more detail. However, the species-specific differences between animal models and VCL patients should be considered, and more trials are needed to come close the fact that, how actually VCL affects the human fertilization potential.

Keywords: Varicocele, Animal Models, Spermatogenesis, Fertilization potential.

I-18: Antioxidant, Anti-inflammatory and Testosterone Therapy Reinforces Spermatogonial Stem Cells Self-renewal in Experimentally-induced Varicocele; Possible Mechanisms

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Background: The varicocele (VCL) has been known as one of the infertility problems in 15-20% of the male population, which severely affects the spermatogenesis via inducing oxidative, inflammatory stresses and suppressing testicular endocrine potential. Thus, the antioxidant, anti-inflammatory and testosterone boosting chemicals (herbal and/or synthetic) have been considered as the alternative therapeutic methods. Thus, the VCL-induced damages can be divided into a-failed endocrine network between Leydig and Sertoli cells, b-the cytokines-induced effects on transcriptional factors and encoding genes, c-the oxidative stress-related molecular changes at cell cycle machinery.

Materials and Methods: To analyze mentioned three mechanisms, the experimental VCL was induced in Wistar rats, then the animals were divided into VCL-sole and antioxidant, anti-inflammatory and testosterone treated VCL-induced groups. Following 2 months, the animals were euthanized and the testicular glial cell line-derived neurotrophic (GDNF), its receptors Gfra1 and C-ret, the encoding active genes of spermatogonial stem cells (SSCs) self-renewal Bcl-6b and Etv5, and genes involving in cell cycle machinery including, Cylin D1, CDK-4, p21, and the inflammatory mediators, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), cyclooxygenases (COX-II) and nitric oxide (NO), and the homeostatic factors heat shock protein70-2 (Hsp70-2), E2f1 expressions, serum levels of testosterone and inhibin B, the testicular total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione peroxidase (GSH-px), superoxide dismutase (SOD), catalase, total thiol molecules (TTM) were investigated, using different RT-PCR, immunohistochemical, western blot and ELISA methods. the germinal cells DNA fragmentation was assessed using TUNEL staining. Moreover, the sperm parameters including, sperm count, viability, motility, DNA integrity, chromatin condensation were assessed. All results were compared between VCL-sole and treatment groups.

Results: Observations revealed that, administrating antioxidant and anti-inflammatory chemicals in association with testosterone boosting agents significantly ameliorates the VCL-impaired Leydig-Sertoli network, amplify the VCL-diminished GDNF, Gfra1, C-ret, Bcl-6b and Etv5 expression, and finally through this mechanism promote the SSCs self-renewal. Moreover, we showed that promoting the testicular endocrine and antioxidant system remarkably down regulates the DNA fragmentation, suppresses the p21 expression, amplifies the Cyclin D1 and CDK-4 expression, and through this mechanism promotes

cell cycle progression in SSCs. More observations revealed a remarkable reduction in inflammatory mediators expression/synthesis/activity in treated groups. the animals in antioxidant and anti-inflammatory chemicals-treated groups exhibited enhanced testicular Hsp70, TAC, GSH-px, SOD, catalase and TTM levels and represented diminished E2f1 and apoptosis indices versus VCL-sole group. Finally, the VCL-treated groups exhibited improved sperm parameters compared to VCL-sole group.

Conclusion: The antioxidant and anti-inflammatory therapies in association with testosterone boosting agents (in sole and simultaneous form of administration) promote the Leydig-Sertoli cells physiologic interactions, which in turn a- amplifies the Sertoli-related niche factors expression/synthesis and affect on SSCs self-renewal, b- downregulates the inflammatory mediators expression/synthesis and affect on SSCs self-renewal, c- reduces DNA fragmentation both at germ cells and sperm levels and amplifies the homeostatic factors Hsp70-2 expression and suppresses the E2f1 protein level and d- improves the sperm parameters resulting in enhanced fertilization potential.

Keywords: Varicocele, Spermatogonial Stem Cells, Self-Renewal, Cell Cycle

Embryology

I-19: Effects of Carnitine and Chromium Co-Supplementation on Body Weight, Metabolic and Genetic Profiles in Women with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective: The aim of this study was to evaluate the effect of the co-administration of carnitine and chromium on body weight, metabolic and genetic parameters in women suffering from PCOS.

Materials and Methods: This randomized, double-blinded, placebo-controlled clinical trial was conducted on 54 subjects, aged 18-40 years old. Subjects were randomly allocated to take either 1,000 mg/day carnitine plus 200 μ g/day chromium as chromium picolinate (n=27) or placebo (n=27) for 12 weeks. Glycemic control and lipid profiles were measured at baseline and after the 12-week intervention. Gene expression related to insulin and lipid metabolism was conducted on peripheral blood mononuclear cells (PBMCs) of PCOS women using RT-PCR method.

Results: Carnitine and chromium co-supplementation, compared with the placebo, significantly reduced body weight (-4.2 \pm 2.4 vs. -1.9 \pm 2.3 kg, P<0.001) and BMI (-1.6 \pm 0.9 vs. -0.2 \pm 0.4 kg/m², P<0.001). Participants who received carnitine plus chromium supplements had significantly lower fasting plasma glucose (β -3.66 mg/dL; 95% CI, -6.44, -0.89; P=0.01), serum insulin levels (β -1.64 μ IU/mL; 95% CI, -2.40, -0.88; P<0.001), homeostasis model of assessment-insulin resistance (β -0.37; 95% CI, -0.55, -0.20; P<0.001), serum triglycerides (β -25.83 mg/dL; 95% CI, -35.37, -16.28; P<0.001), VLDL- (β -5.16 mg/dL; 95% CI, -7.07, -3.25; P<0.001), total- (β -18.16 mg/dL; 95% CI, -26.39, -9.92; P<0.03), LDL- (β -12.79 mg/dL; 95% CI, -21.61, -3.97; P=0.005) and total-/HDL-cholesterol ra-

tio (β -0.36; 95% CI, -0.72, -0.009; $P=0.04$), and higher quantitative insulin sensitivity check index (β 0.96; 95% CI, 0.77, 1.15; $P<0.001$) compared with the placebo. Moreover, carnitine and chromium co-supplementation upregulated gene expression of peroxisome proliferator-activated receptor gamma (PPAR- γ) ($P=0.02$) and low-density lipoprotein receptor (LDLR) ($P=0.02$) compared with the placebo.

Conclusions: Overall, the co-administration of carnitine and chromium for 12 weeks had beneficial effects on body weight, glycemic control, lipid profiles except HDL-cholesterol levels, and gene expression of PPAR- γ and LDLR among women with PCOS.

Keywords: Carnitine, chromium, insulin metabolism, lipid profiles, polycystic ovary syndrome

I-20: Biomarkers and Key Considerations for Single Embryo Transfer: Which Embryo to Transfer Based on Early Embryo Assessments?

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Continuous embryo monitoring (CEM) unquestionably made life in the laboratory more comfortable, apparently even for the embryo, but mostly for the embryologist. However, it did not make things more comfortable for the clinicians that may not understand its outstanding contribution to embryology. At the present time, we face opposition from those completely against CEM, gynecologists and embryologists that have never used this technology, with absolutely no idea of its applications, daily routine work, or clinical experience. Instead their opinions are based on a few words read in some of the latter reviews or meta-analyses. Maybe this affirmation is exaggerated, but anyone without experience and only a small number of concepts taken from the scientific literature cannot become an opinion leader of any technology or specialty and even more make decisions related to groups of clinics or national societies. Then, what else can experts do? We can keep working hard and provide more convincing data. We may be partially agreed in affirming that CEM remained in search of an indication. In the last few years, many authors decided to investigate whether application of CEM reveals new algorithms related with embryo selection. This research started with a deep analysis of the fertilization process moving forward to the cleavage stage, including multinucleation, symmetry or synchrony, being those potentially able to predict embryo quality at latter stages of development. But even more, the analysis of the final steps of development (blastulation) may reveal important features that could condition the outcome as collapse, expansion, inner cell mass diameter or even final blastocyst diameter.

We must take into consideration the conflicting data from the publications reported until now. They were coming from different laboratories, and serve to emphasize the fact that many patient and laboratory variables affect embryo kinetics. Selection algorithms may require extensive testing by different laboratories and in-house refinement, a new question that remains to be elucidated. We may also point out the need for prospective validation studies with day 5/6 blastocysts selected for transfer based on kinetic endpoints, but, those studies are extremely difficult to be undertaken. Cost, patient recruiting, and ethical issues are hurdles in front of a researcher seeking answers to the big questions concerning TLM and reproductive medicine.

We may also want to know the chances of implantation of an euploid embryo by analyzing their morphokinetics. In an environment of increased PGS demand, in which more euploid embryos will become suitable for transfer, CEM will provide more chances to select the perfect euploid blastocyst.

I-21: New Strategies for Diagnosing Embryo Implantation Potential

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I-22: Regulation of Sperm Development by Zinc: Insights from Quantitative Subcellular X-Ray Fluorescence Imaging

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This talk will focus on the zinc fluxes that regulate sperm activation. Quantitative single cell X-ray fluorescence microscopy at APS beam lines and the Bionanoprobe at Argonne National Laboratory reveals a critical regulatory role changes in zinc content and subcellular localization. Recent applications of synchrotron based X-ray fluorescence microscopy (XFM) are at the heart of a number of breakthroughs in our understanding of the most fundamental aspects of biology. In conjunction with label-free, high-resolution ptychographic imaging, XFM is greatly enhancing the ability of chemists and biologists to interrogate chemical changes in subcellular compartments during developmental processes. Our studies of zinc fluxes in sperm include live cell studies in confocal fluorescence experiments, ICP-MS and radiotracer analysis. We find that full sperm function in fertilization requires the movement of zinc ions between subcellular compartments: millions of zinc ion must be taken up by sperm and accumulate in the nucleus. These movements of zinc must occur in a tightly regulated time over minutes to hours and sperm cells that do not undergo these fluxes are much less successful in fertilization of mouse eggs *in vitro*. This work reveals that fluctuations in the concentration and binding sites of zinc, like calcium and phosphorus, act as conductors of information in biological signaling networks. As sensitivity and resolution of XFM methods increase, we anticipate that quantitative imaging of the fluxes of other abundant inorganic cofactors, such as copper, potassium, sodium, iron and manganese will likewise reveal new mechanisms in biology.

I-23: New Regulators of Meiotic Progression: The Zinc Spark and Zinc Flux

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Egg quality can be gauged using a number of genetic, biochemical and physiological metrics, however most of these are invasive or require repeated observations of the zygote as it matures into an embryo. We have recently examined an array of quantitative inorganic markers of egg quality and have found a promising extracellular indicator of egg quality, namely the quantity of zinc released at the time of fertilization. All cells, including gametes carefully maintain a limited ensemble of transition metals (i.e. zinc, copper, iron, manganese, etc.) the concentrations of which are collectively referred to as the metallome of the cell. It is becoming increasingly apparent that temporal fluctuations in the zinc metallome, as well as changes in labile zinc concentrations in subcellular compartments, play essential signaling roles that control oocyte maturation, fertilization and early embryonic development. Using single cell quantitative X-ray and small molecule fluorescence microscopy, we show that both gametes, i.e. sperm and egg, must undergo dramatic translocations and/or exocytosis of zinc in short time frames to ensure successful fertilization. In this talk, the cellular mechanisms and origins of the fertilization-induced zinc exocytosis events known as 'zinc sparks'. Quantitative accounting of amount of zinc released per egg, the evolutionary conservation and the roles of extracellular zinc, and ultimately the functions of zinc fluxes in sperm will be discussed. These results have led us to test whether inorganic markers such as zinc may serve as a robust indicator of egg quality.

I-24: The Surprising Role of Zinc in Oocyte Maturation and Embryo Progression

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Egg activation refers to events required for transition of a gamete into an embryo, including establishment of the polyspermy block, completion of meiosis, entry into mitosis, selective recruitment and degradation of maternal mRNA, and pronuclear development. Here we show that zinc fluxes accompany human egg activation. We monitored calcium and zinc dynamics in individual human eggs using selective fluorophores following activation with calcium-ionomycin, ionomycin, or hPLC ζ cRNA microinjection. These egg activation methods, as expected, induced rises in intracellular calcium levels and also triggered the coordinated release of zinc into the extracellular space in a prominent "zinc spark." The ability of the gamete to mount a zinc spark response was meiotic-stage dependent. Moreover, chelation of intracellular zinc alone was sufficient to induce cell cycle resumption and transition of a meiotic cell into a mitotic one. Together, these results demonstrate critical functions for zinc dynamics and establish the zinc spark as an extracellular marker of early human development.

I-25: Oncofertility: from Bench to Bedside to Babies

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Facing a cancer diagnosis at any age is devastating. However, young cancer patients have the added burden that life-preserving cancer treatments, including surgery, chemotherapy, and radiotherapy, may compromise their future fertility. The possibility of reproductive dysfunction as a consequence of cancer treatment has a negative impact on the quality of life of cancer survivors. The field of oncofertility, which merges the clinical specialties of oncology and reproductive endocrinology, was developed to explore and expand fertility preservation options and to better manage the reproductive status of cancer patients. Fertility preservation for females has proved to be a particular challenge because mature female gametes are rare and difficult to acquire. The purpose of this presentation is to provide a comprehensive overview of how cancer treatments affect the female reproductive axis, delineate the diverse fertility preservation options that are currently available or being developed for young women, and describe current measures of ovarian reserve that can be used pre- and post-cancer treatment.

I-26: A Bioprosthetic Ovary Created Using 3D Printed Microporous Scaffolds Restores Ovarian Function in Sterilized Mice

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Background: Emerging additive manufacturing techniques enable investigation of the effects of pore geometry on cell behavior and function. Here, we 3D print microporous hydrogel scaffolds to test how varying pore geometry, accomplished by manipulating the advancing angle between printed layers, affects the survival of ovarian follicles.

Materials and Methods: 30° and 60° scaffolds provide corners that surround follicles on multiple sides while 90° scaffolds have an open porosity that limits follicle-scaffold interaction. As the amount of scaffold interaction increases, follicle spreading is limited and survival increases.

Results: Follicle-seeded scaffolds become highly vascularized and ovarian function is fully restored when implanted in surgically sterilized mice. Moreover, pups are born through natural mating and thrive through maternal lactation.

Conclusion: These findings present an *in vivo* functional ovarian implant designed with 3D printing, and indicate that scaffold pore architecture is a critical variable in additively manufactured scaffold design for functional tissue engineering.

Keywords: Biomaterials, preclinical research, translational research, fertility

I-27: New Communication Strategies for Reproductive Science and Medicine

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I-28: A Microfluidic Culture Model of the Human Reproductive Tract and 28-Day Menstrual Cycle**Woodruff TK**

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Ethics and Reproductive Health**I-29: Precision Medicine in COH: Ethical Perspectives****Ommani-Samani R**

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Doctors always wanted to find ways to make distinction between their patients to choose the most effective treatment for them. There have been diagnostic tools to help the doctors make their medical practice more "personalized". Developments in genetics opened a new door to physicians by introducing some genes that are responsible for drug resistance, so evaluations moved to molecular and cellular tests for distinguishing between patients according to their drug resistance and sensitivity called: "precision medicine".

Controlled ovarian hyperstimulation (COH) has been used for oocyte retrieval in assisted reproduction using high doses of hormones to stimulate the ovaries and get as many oocytes as can be. Reaching to several good quality oocytes is the aim of COH that cannot be achieved in many patients. It is imagined that maybe genetic differences are responsible for resistance to some COH protocols and sensitivity to the others. For this reason, precision medicine researches have been conducted many studies to find out genetic factors encoding resistance to COH drugs.

There are several ethical issues about precision medicine that should be considered. Cost-effectiveness is very important especially in costly procedures including genetic tests. It is needed to pursue a protocol indicating when using these techniques are cost effective for the patients. For example, for the first cycle? Or, after first or second failure? Or when the patient is marked as recurrent failure? Availability of the test is also important ethical issue. Genetic testing is not available everywhere and need sophisticated equipment. Health care coverage of these tests is under question, so, they will be available only for rich people. Commercial usage of the techniques brings "over use" and "conflict of interest".

It can be concluded that precision medicine is promising for the future of COH but needs to prove efficacy, cost effectiveness and feasibility and guidelines should be designed to prevent any misuse or conflict of interest.

Keywords: Precision Medicine, COH, Ethics, Conflict of Interest, Cost Effectiveness

I-30: Fertility Preservation: Legal and Ethical Issues**Shariati Nasab S**

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Fertility preservation (FP) is an expression for saving reproductive tissues, gametes or embryos for future usage that maybe in that time, gametes cannot be reached or have low quality. There are two main kinds of fertility preservation: a. with medical reasons (like cancer and chemotherapy) and b. with social reasons (advancing age and ovarian failure). Each one has its own ethical and legal issues.

a. With medical reason: any treatment (like chemotherapy or radiotherapy) which can damage reproductive system may induce future infertility. So, gametes or embryo should be cryopreserved for preserving the possibility of future child bearing. There are some factors that should be ethically considered: prognosis and life expectancy, type of cancer and sensitivity to hormones, possibility of transmission of cancer to the child, necessity of starting the chemo at once, puberty of the patient, disposition of cryopreserved materials, posthumous reproduction.

b. With social reasons: when women get older than 40, fertility ability drops dramatically even if menopause does not happen. For this reason, some women request for cryopreservation of their oocytes for future use. In these cases, also there are ethical issues like: preventing misguides and promising to the patients about their future pregnancy, changing the trend of the society, people right to know about this service and advertisement.

There are some legal points also in these procedures specially in Iran: obligation of oncologist to inform their patient preventing their future infertility as physician responsibility, girls' virginity and legal consequences, informed consent with full information considering that some of FP methods are still in research phase. It can be concluded that some law and guidelines must be passed to clarify the physicians' responsibilities and also ethical considerations for fertility preservation.

Keywords: Fertility Preservation, Legal, Ethical, Responsibility, Misguide

Female Infertility**I-31: Nonsurgical Treatment of Endometrium****Aflatoonian A**

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Ovarian endometrioma is detected in 17-44% of patients with endometriosis, and may be related to infertility, dysmenorrhea and chronic pelvic pain. However, its negative effects on fertility potential are poorly known; the harmful outcomes can appear as both mechanical impact and biochemical effects. The conventional approach to the ovarian endometrioma is laparoscopic excision of the cyst capsule. As the surgical treatment on endometrioma could be detrimental to ovarian reserve and subsequently adversely affect IVF/ICSI reproductive outcomes, evidence-based studies suggest surgical excision in the case of unilateral symptomatic or large (>3 cm) endometriomas among patients with good ovarian reserve. Ovarian cystectomy can lead to decreased ovarian reserve due to the removal of healthy ovarian tissue adjacent to the cyst wall along with excessive coagulation to the ovary for hemo-

stasis. These findings have led to a shift of opinion toward a more conservative approach in the treatment of endometrioma. An alternative less invasive approach to endometrioma is aspiration, or sclerotherapy with sclerosing agents include: ethanol, tetracycline, synthetic interleukin-2 and methotrexate. Regarding ethanol sclerotherapy, in a randomized clinical trial, an interventional group of 20 patients underwent transvaginal ethanol sclerotherapy for recurrent ovarian endometrioma were compared to 20 women with ovarian endometrioma without sclerotherapy. The recurrence rate after 6 months was 20% in the case group. The clinical pregnancy rate was (33.3% vs. 15%) in the ethanol and control group respectively ($P=0.616$). The fertilization rate emerged 63.06% in the study group vs. 60.38% in control women ($P=0.57$). The implantation rate was reported 12.9% vs. 7.5% in the study and control groups respectively ($P=0.52$). Overall, the data pointed to a better trend toward the ethanol sclerotherapy group. Similarly, a recent meta-analysis indicated that ethanol sclerotherapy for ovarian endometrioma may be considered in symptomatic women who plan to conceive. Moreover, in an unpublished research 37 patients with ovarian endometrioma were compared regarding ethanol retention or ethanol aspiration during sclerotherapy. Chemical pregnancy rate was 50% and 56% in ethanol retention and ethanol washing group respectively. 55% of pregnancies were achieved during ART and 45% get pregnant spontaneously. Therefore the duration of ethanol inside the endometrioma seems to be important. Long exposure of endometriosis cells to ethanol by means of prolonged washing time or in situ retention is likely to achieve complete inactivation of these cells. In conclusion, since endometrioma is a multifactorial disease, therefore a multifactorial approach should be applied. The increasing trend toward non-surgical treatment is encouraged. Surgical treatment should be limited to younger women with good ovarian reserve, unilateral symptomatic endometrioma. Nonsurgical treatment is a safe method for patients with bilateral endometrioma, low ovarian reserve and previous failed surgery. Direct ART or sclerotherapy.

I-32: Breast Cancer and Ovulation Induction Treatments

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Background: This study was performed to determine whether the use of ovulation induction drugs in treatment of infertility have a significant effect on the risk of breast cancer.

Materials and Methods: This case control study (928 cases, 928 controls), was performed in the gynecology and oncology clinics of Shahid Beheshti University of Medical Sciences between 2011 and 2013. Data were collected via in-person interviews using a questionnaire, which included demographic and gynecologic information. Statistical analysis was performed using SPSS statistics software version 20 (IBM Corp).

Results: The use of ovulation induction drugs was not signifi-

cantly associated with an increased risk of breast cancer (odds ratio [OR], 1.13; 95% confidence interval [CI], 0.7-1.855) among women with infertility (OR, 1.28; 95% CI, 0.8-1.95).

Conclusion: We observed no statistically significant relationship between infertility and ovulation induction drugs with the risk of breast cancer, except for significant increases in the risk of breast cancer among patients who had used fertility drugs for >6 months.

Keywords: Breast Cancer, Clomiphene, Gonadotropins, Infertility, Ovarian Stimulation

I-33: Decreased Ovarian Reserve: any New Hope?

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While diminished ovarian reserve (DOR) predicts decreased ovarian response to stimulation, it does not necessarily foretell about the fecundity cycle. According to Bologna's criteria laid down by the European Society of Human Reproduction and Embryology, old age, abnormal ovarian reserve tests such as antral follicle count (AFC) and anti-mullerian hormone (AMH) as well as prior suboptimal response to stimulation are the main factors representing DOR. Unfavorable response to maximal stimulation on two previous occasions may also represent DOR. Among the ovarian reserve tests, AMH and AFC are the most predictive values for DOR. Factors which may give rise to DOR include environmental factors, autoimmune or metabolic disorders, infections, genetic abnormalities, and iatrogenic causes (such as smoking, chemotherapy, radiation and gynecologic surgeries). Besides, studies have proposed endometriosis as a key contributor to DOR and hence emphasized on its proper management to prevent additional damages leading to compromised fertility. In summary, DOR is found to be a clinical challenge in the practice of fertility care with controversial countermeasures to prevent or treat the condition. Nevertheless, some promising measure such as: oocyte, embryo and tissue cryopreservation, ovarian transplantation, dietary supplementation and the transfer of mitochondria have offered hopes towards ameliorating the burden of DOR. This review attempts to discuss DOR from different perspectives and summarize some existing hopes in clinical practice.

I-34: Genetics of Male Infertility

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Background: Our objective is to detect the frequency and types of major genetic abnormalities of idiopathic non-obstructive azoospermia (NOA) to give appropriate genetic counseling before assisted reproductive techniques (ART) in Middle East and to compare the frequencies with other regions of the world.

Material and Methods: A total of 880 Middle Eastern patients with NOA were recruited in this multicenter study for genetic evaluation prior to use of ART. Karyotyping was performed on

peripheral blood lymphocytes according to standard G-banding methods, polymerase chain reaction (PCR) was performed to screen the microdeletions in the AZF region of the Y chromosome.

Results: The present study shows that the total prevalence of genetic abnormalities is 28.41 %, including 184 patients (20.91 %) with chromosome disorder and 66 patients (7.5 %) with Y chromosome microdeletions. The most prevalent chromosome abnormality is Klinefelter's syndrome, which includes 161 patients (18.3 %), 7 patients had XX reversal male sex (0.8 %), 2 patients had 47XYY (0.23 %) and 2 patients had 45XO/46XY (0.23 %). Structural abnormalities occurred in 12 patients (1.36 %).

Conclusions: The high prevalence of genetic abnormalities (28.41 %) in our study strongly suggests the need for routine genetic testing and counseling prior to assisted reproduction in such population with idiopathic infertility, as a result may help determine the prognosis, as well as the choice of ART. Moreover it allows specific pre-implantation genetic testing to minimize the risk of transmitting genetic defects to offspring.

Keywords: Genetic Abnormality, Non-Obstructive Azoospermia, Microdeletion

I-35: Inherited Thrombophilia and Recurrent Pregnancy Loss

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Background: RPL is a multifactorial condition, defined as two or more consecutive pregnancy losses and affecting 1-5% of reproductive-age woman. After chromosome abnormality, thrombophilia is one of the most important genetic factors that could cause RPL. However, the etiology of RPL remains unknown in ~50 % of cases. Although numerous studies are available in literature, thrombophilia rate seems to vary from study to another due to different selection criteria and ethnicity of patients.

Our objective is to evaluate the prevalence of thrombophilic gene mutations in Syrian women with recurrent pregnancy loss (RPL) compared with women who had uneventful pregnancies.

Materials and Method: In this is case-control study, the frequency of thrombophilic gene mutations were determined in a consecutive series of 100 women referred for evaluation of recurrent spontaneous pregnancy loss (case patients) between October 2012 and October 2015. The control group included 100 women from the same ethnic background and with at least one successful pregnancies and no history of pregnancy loss, which matched by age with patients.

100 women with RPL (who have two or more pregnancy losses) were recruited in this study, compared with 100 women without adverse pregnancy outcome. Genotyping of thrombophilic gene mutations were carried out by amplification Refractory Mutation System- PCR (ARMS-PCR) method after DNA extraction.

Results: This study has shown that Factor V Leiden (FVL) is significantly associated with RPL compared with controls (P values was 0.0025). And there were no statistically significant differences in the prevalence of methylenetetrahydrofolate reductase MTHFR (C677T and A1298C), prothrombin (G20210A) mutation and other mutations between RPL patients and controls. It is of interest to note that FVL constitutes

a major risk factor if RPL is considered in comparison with controls [Odds Ratio were 4.13(CI: 1.43-5.85)].

Conclusion: Thrombophilia accompanied with FVL may plays a role in pathophysiology of RPL, suggesting that attention should be directed at screening women with recurrent pregnancy loss for detection of FVL.

Keywords: Thrombophilia, Recurrent Pregnancy Loss, Factor V Leiden, MTHFR, Prothrombin Mutation.

I-36: Endometriosis and ART

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We recommend assisted reproductive technology (ART) to endometriosis patients with old ages and high duration of infertility, especially when other treatment have been unsuccessful and also when we have other infertility causes such male Factor Infertility and any problems in tubal function are existence.

In infertile woman with endometrioma larger than 3 cm there is no evidence that cystectomy prior to treatment with ART improves pregnancy rate.

But if these cysts produce severe pain or some problem for ovum pick up, We can do cystectomy but must know about risk of reduced ovarian function after surgery.

The use of GnRH analog pituitary suppression with the so-called "long protocol" is the gold standard among the COH protocols. It should be used as the first line of treatment in women with good ovarian reserve.

ESHRE guideline recommends GnRH α 3-6 month before ART, to improve pregnancy. But in special patient with low ovarian reserve we can use Antagonist Protocol. Antibiotic prophylaxis is recommended in these patients during oocyte retrieval.

Most studies show that endometriosis affects number of retrieved oocyte but not embryo quality or pregnancy outcome irrespective of the presence of an ovarian endometrioma. Miscarriage and obstetric complication are higher in patient special with severe endometriosis.

ART did not exacerbate the symptoms of endometriosis or negatively impact on quality of life in women with endometriosis as compared with disease-free women.

I-37: Management in POI Patients

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In IVF, poor response to controlled ovarian stimulation (COS) represents an important issue, which may affect about 10% of the infertile women. Based on the Bologna criteria at least two of the following characteristics must be present to define "a poor responder patient": advanced maternal age (>40 years)

and/or scarce response to a previous conventional stimulation (≤ 3 oocytes) and/or reduced ovarian reserve (antral follicle count, AFC $< 5-7$ follicles, and/or AMH < 1.1 ng/ml). Currently, the management for this group is not elucidated yet, and the prognosis is highly dependent more on patients' specific characteristics, rather than upon the COS protocol chosen. Many protocols like standard antagonist, minimal stimulation and DuoStim protocol can be clinically applied to this population of patients of poor prognosis to get more and higher quality eggs and embryos. Several adjuvant treatments have been proposed to determine the factors interfering with follicular output ratios and increased ovarian response to gonadotropins, but consensus has not been reached around a strategy to treat poor responders undergoing IVF. There are limited data supporting the isolated use of adjuvants such as DHEA, rLH, hCG or letrozole and transdermal testosterone to improve the outcomes of IVF cycles in poor responders. In this review, we intend to provide an update about the IVF management of poor responders, as well as to describe and evaluate the use of novel strategies to increase pregnancy rates.

Keywords: Poor Responder, IVF, Protocols

I-38: Importance of Toxins in Repro-Medicine; Focusing on IVF Outcomes

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In vitro fertilization (IVF) is an assisted reproductive technology (ART) in which the ovum is fertilized by sperm outside of the physical structure. The zygote is cultured in growth medium for approximately 5 days and the resulting embryo or blastocyst is transferred back into the women. The first IVF pregnancy was accomplished in 1978 and since then, IVF and its variant intracytoplasmic sperm injection (ICSI), has become the main form of ART used to treat infertility for both males and females. The use of ARTs such as IVF is increasing. Consequently, it is highly important to know all factors that can affect the success rate of IVF, especially due to the emotional costs, time and money invested into treatment cycles. The lifestyle factors, including cigarette smoking, alcohol use and nutritional habits influence oocyte production, fertilization rates, pregnancy and pregnancy loss.

Reproductive maturation and function are similarly influenced by early-life consequences. This should not be surprising, because the primordial follicle pool is established early in life and is thus vulnerable to early-life events. Outcomes of clinical and experimental studies have indicated that early-life adversity is associated with a decline in ovarian follicular reserve, changes in ovulation rates, and altered age at onset of puberty.

Female reproduction is regulated by hormones and is susceptible to the effects of exposure to endocrine disrupting chemicals. Disruptions in female reproductive functions by endocrine disrupting chemicals may result in subfertility, infertility, improper hormone production, oestrous and menstrual cycle abnormalities, anovulation, and early reproductive senescence.

Furthermore, fertilization and embryo development *in vitro* have the potential to introduce stresses which cannot only impair embryo development in the laboratory, but also which can have downstream effects after transfer. The current subject area will reveal the toxins impact on fertility and treatment outcomes using systematic reviews and Meta analysis.

I-39: Endometrial Transcriptome and Proteome Profiles in Women with Impaired Fertility-Further Advancements

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Implantation in humans is a complex process. Over the past decade, several genes and gene products that may participate in this process have been identified in secretory phase endometrium. Improvements in assisted reproduction techniques such as IVF and ICSI mean that for the majority of couples successful fertilisation and embryo replacement is achieved. However up to 90 % of embryos transferred to the uterus fail to implant, and for some couples successful implantation is never achieved. Understanding the factors affecting uterine receptivity is a major challenge for those seeking to improve treatment for infertile women. In this study, I determine the transcript profile of receptive phase endometrium obtained from fertile and unexplained infertile women, in the hope that they may lead to improved treatments for this enigmatic disease. I use microarray technology to determine the levels of thousands of transcripts in a timed endometrial biopsies (LH+5 to LH+10). Through the application of Class Prediction Tool, specifically designed for the analysis of extensive microarray data sets, I identify transcripts that are present at significantly different levels in receptive phase endometrium, from women with and without unexplained infertility. I discover a number of transcripts that have not previously been identified in either the endometrium or unexplained infertility, and report two new transcripts that may be important in the pathology of unexplained infertility. I propose that Serum- and Glucocorticoid- inducible Kinase-1 is required in the endometrium during the secretory phase of the menstrual cycle for embryo implantation and decidualisation. I determine protein signatures that can differentiate between normal endometrium and endometrium with persistent reproductive failure and potentially be used as a diagnostic tool. I applied SELDI-TOF-MS technique to determine the protein profile of receptive phase endometrium obtained from fertile and infertile women. I identified four peaks that were present at consistently significantly different levels between patient groups. I identified a novel protein Apolipoprotein A-I that was present at significantly different levels between endometrium of fertile and infertile women during the implantation window. I suggest that Apolipoprotein A-I may be involved in the acquisition of endometrial receptivity. These findings offer novel insights into the regulation of endometrial receptivity and its potential pathologies. The identification of these new transcripts and proteins in the endometrial system are a step towards the development of improved treatments for unexplained infertility.

I-40: Social Fertility Preservation

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Fertility preservation are increasing for non-medical reason

in some developed countries. Women are proposing to delay motherhood for some personal problem such as education and career or absence of partner or unstable relationship. Therefore women are offered the possibility of freezing eggs until they wish to have a baby. But they should know, all complication of IVF cycle and health risks to the child and themselves. The success rates depend on the number of mature oocytes and expertise of the center. As a conclusion social freezing merely postpones social problems not solving problem.

I-41: Developing The Concept of iCOS: A Path toward Success in ART

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I-42: ART Challenges and The POR Patients: Aetiologia and Management Developing the Concept of iCOS: A Path toward Success in ART

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I-43: Role of Ultrasound in Infertility: From Diagnosis to Treatment

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Ultrasound has an essential role in infertility. It has a key part in diagnosis, assessment, evaluation and treatment of infertility. The use of 3D ultrasound and Doppler has allowed better imaging. In the presentation, the history of US in infertility and the different pathologies will be reviewed briefly. The remaining part of the presentation will be focused on transvaginal ultrasound hygiene, and the training and accreditation policies for US performance in some countries.

I-44: In Vitro Ovarian Follicle Culture

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I-45: Uterus Transplantation: An Overview

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Today, and by advent of ART, most infertile couples are treated successfully. However absolute uterine factor infertility (AUI) is so far untreatable. By far, the two feasible solutions are either gestational surrogacy or adoption. The possibility of gestational surrogacy or adoption is not accessible in many countries due to legal, religious or ethical consideration. Uterus transplantation (UTx) is the only quality of life enhancing method as well as a life-giving transplantation in women with AUI. After 12 years of research, the first human case of UTx was performed in Saudi Arabia in 2000, and the first baby resulting from a transplanted uterus was born in 2014 in Sweden. In a uterus transplantation procedure, there are two options for uterus donors, living or deceased, both of which have advantages and disadvantages. The candidates of uterus donor & recipient go through a detailed work up prior to the transplantation regarding both the physical and psychological and all laboratory tests. Ovarian stimulation, ART and embryo freezing should be performed one year before transplantation. UTx surgical procedure including procurement of the uterus (Radical Hysterectomy including preservation of long vascular pedicles up to internal iliac vessels, round and uterosacral ligaments, and a flap of bladder peritoneum). This part takes about 14 hours. Immunosuppression must be induced at this point. The next procedure is anastomosis of all preserved part of the uterus to recipient. Anastomosis takes 5 hours. From the ethical point of view, UTx is highly experimental procedure, not life-saving, is an ephemeral transplant, needs immune-suppression, and there is also a debate concerning the application of this option in countries or regions that gestational surrogacy is permitted.

I-46: Adenomyosis and Reproductive Outcome

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Adenomyosis is defined as the presence of endometrial glands in the myometrium. Its prevalence is 20%, the main sign and symptoms are: pelvic pain / AUB / infertility and poor outcome, there is two different types of the disease: focal / diffuse, there is three different types: internal / external / adenomyoma. The best ways to diagnose the disease is: histopathology / MRI and 3D and 2D vaginal ultrasound. The effects of adenomyosis on reproductive outcome is in the field of obstetrics like: increase preterm birth / increase PROM and increase IUGR, and also in the field of infertility life: impaired tubal transfer / decreases sperm function / decrease implantation and altered uterine contractility. Also there is increase in early pregnancy loss and decrease live birth rate. For the treatment / we can suggest pretreatment with GnRha before IVF. Some studies had good results with surgery before IVF / but it needs more researches.

I-47: Bad Teacher or Bad Student: toward Pragmatic Basis of Immunologic Recurrent Pregnancy Loss

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Copious anatomical, genetic, endocrine and thrombophilic disorders have been evoked to explain recurrent reproductive failure; i.e. recurrent pregnancy loss (RPL) or repeated implantation failure (RIF). Based on self/non-self discrimination theory explaining the capacity of immune system to recognize and reject any antigen-bearing foreign material that enters the body, immunological etiologies have also been considered as one the central basis of RPL and RIF. Besides a limited set of autoimmune disorders associated with RPL, literature is replete with evidence highlighting the involvement of allo-oriented immune cells and mediators in RPL and RIF. Nonetheless, it is yet an open question whether immunological imbalance linked to RPL or RIF is intrinsic in essence or resulted from dysregulation of immune network. There is a very balanced and finely-tuned network of immune mediators and cells working in an amazing harmonic way that successfully governs all aspects of reproduction from fertilization to implantation, development and finally parturition. Accordingly, if such regulatory network reverts to its pre-pregnancy functional state with the capacity to discriminate self from non-self, there will be a contrasting force against fetal development. In my personal view, it is the dysregulated nature of endometrial immune network and not necessarily intrinsic dysfunctioning of immune cells that is responsible for immune-mediated RPL or RIF. In this context, growing pieces of evidence suggest that endometrial mesenchymal stem cells (eMSCs) play a pivotal role in such immunomodulation processes, by phagocytosing paternal antigen and antigen presentation, orchestrating the immunological responses at the fetomaternal interface through secretion of various cytokines and chemokines and instructing endometrial immune cells. During the past couple of years, we provided compelling evidence showing that eMSCs regulates phenotype and function of immune cells and shape them to a pregnancy-friendly phenotype. These results imply that dysfunctioning of immune cells in such pregnancy-related disorders as RPL and RIF may not be considered as an intrinsic defect, rather it may stem from misleading information released from their instructor, endometrial stromal stem cells.

Key words: Recurrent Pregnancy Loss, Repeated Implantation Failure, Stem Cells, Immune Dysregulation

Genetics

I-48: Genetics and Molecular Characterization of The Multiple Morphological Abnormalities of The Sperm Flagella (MMAF) Syndrome

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Background: Male infertility is a complex disorder that affects more than 20 millions men worldwide and has become a global concern that affects many aspects of human life. 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 very 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. Multiple morphological abnormali-

ties of the flagella (MMAF), previously described as “dysplasia of the fibrous sheath”, “short tails” or “stump tail syndrome”, is one of the most severe forms 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). The abundance of potential candidate genes makes identification of pathogenic mutations difficult and complex. 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. 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. In this study, Whole Exome Sequencing (WES) has led to the identification of new genes involved in MMAF and exploits the WES data to the benefit of the patients.

Materials and Methods: In our study, we analyzed 78 MMAF patients using WES and showed that in addition to mutations in DNAH1, mutations in CFAP43 and CFAP44, two tryptophan-aspartic acid repeat (WDR) containing proteins, and also in CFAP69 are responsible for MMAF syndrome. The effect of all candidate variants was confirmed by RT-PCR and immunohistochemistry. Most importantly, we investigated the role 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: Using this original approach we demonstrate the importance of WDR proteins for the axonemal structure of the flagella throughout evolution. Overall, DNAH1, CFAP43, CFAP44, and CFAP69 mutations were identified in 30% of the analyzed sporadic subjects (24 out of 78 patients) originating from North Africa, Europe, and the Middle East.

Conclusion: Altogether, our results underline the global importance of these 4 genes in the MMAF syndrome and will improve the genetic diagnosis efficiency of infertile MMAF patients. In our study, WES revealed that CFAP43, CFAP44, CFAP69, and DNAH1 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 the MMAF phenotype.

Keywords: Male Infertility, Genetic Diagnosis, Exome Sequencing, Gene Mutations, MMAF

I-49: Why, When and for whom PGD and PGS May be Beneficial

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Whole chromosome aneuploidies represent the single most important cause for implantation failure and miscarriage in humans. For this reason, embryo genetic assessment during IVF cycles can improve the efficacy of ART treatment. The category of patients at higher risk of producing abnormal embryos, and therefore could benefit most from PGT-A, is sub-fertile women of advanced maternal age (AMA; commonly defined as >35 years). This is due to the exponential increase in incidence of chromosome mis-segregation events in women between the ages of 35 and 43 years. PGT-A is also offered to patients with

a history of recurrent pregnancy loss (RPL; commonly diagnosed after three miscarriages) or repeated implantation failure (RIF; over three failed embryo transfers), and couples presenting severe male factor. Additional indications for PGT-A have been recently proposed including a history of genetically abnormal pregnancy, previous chemo-radiotherapy, reduced embryo quality or the intention to undergo elective single embryo transfer (eSET) during IVF treatment. However, a general agreement on these additional indications is still missing.

Preimplantation genetic testing for monogenic disorders (PGT-M) is indicated for those couples at high risk of conceiving a child affected by an inheritable monogenic condition. Theoretically, it can be used for all genetic disorders for which a precise molecular diagnosis or a definite family marker linkage is present. According to the latest ESHRE PGD consortium data, the most common indications for autosomal recessive disorders are cystic fibrosis, spinal muscular atrophy, and hemoglobinopathies, which involve the presence of two mutated copies from each healthy carrier parent. For the autosomal dominant conditions, the presence of one mutated copy of the gene is sufficient for a person to be affected. Myotonic dystrophy type 1, neurofibromatosis, and Huntington's disease are the most frequently tested conditions. Pathologies with X-linked recessive transmission, are inherited by healthy carrier mothers who transmit them to their children. The main pathologies for which PGT-M is performed are Duchenne muscular dystrophy, Becker muscular dystrophy, Fragile X syndrome and Haemophilia. Several studies have reported the clinical use of simultaneous detection of monogenic and chromosomal disorders using different technologies. This combined analysis allows the avoidance of both embryos carrying inheritable mutations and embryos with an abnormal karyotype, thus maximising the beneficial effect of PGT.

I-50: Epigenetic Regulation of Coding and Non-coding RNA Expression During The 1st Wave of Spermatogenesis

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Objective/Background: Spermatogenesis is a highly regulated process in which undifferentiated spermatogonial stem cells differentiate to form highly specialized sperm cells capable of fusing with the ovum to form a zygote. This is achieved through tightly controlled regulation of gene expression which depends on transcriptional, post-transcriptional, translational and epigenetic mechanisms. Using the first wave of spermatogenesis in mouse as a model, we profiled the transcriptome, mirnome and histone methyl marks in relation to gene expression in the testis during initiation of spermatogenesis. We integrated these three big data sets to generate a network of H3K4Me3 marks on the promoter/ transcription start sites (TSS) and its impact on the expression miRNAs and their target transcripts. Further, we evaluated the effect of silencing of a Meisetz, a known meiosis specific H3K4 methyl transferase, in germ cell line to assess the validity of the networks thus generated.

Materials and Methods: Mice of age groups Day 8, Day 16 and Day 24 were used for this study. RNA extraction was done using miRNAeasy kit (Qiagen). miRNA microarray was done using mercury LNA array, Version 11.0 (Exiqon) containing capture probes targeting all miRNAs for human, mouse or rat

(miRBASE 13.0, Exiqon). Whole transcriptome analysis was done on a GeneChip 1.0 ST array (Affimetrix) containing 28853 well-annotated genes. For chromatin immunoprecipitation (ChIP), isolated genomic DNA was crosslinked with bound proteins, sheared and immunoprecipitated using anti-H3K4Me3 antibodies. The ChIPed fraction was decrosslinked and libraries were prepared, which were subjected to next-generation sequencing on HiSeq 2500 (Illumina). The input DNA (Non ChIP'd genomic DNA) was used as the control. Quality check of the raw reads was performed using SeqQCv2.2. Filtered high quality reads from INPUT and IP files were aligned to reference genome (mm10) using Bowtie-0.12.8 alignment tool using Mouse (*Mus musculus*) reference genome downloaded from UCSC and indexed using Bowtie-build. These three data sets were integrated using Cytoscape Version 2.8.3 (Cytoscape Consortium).

Results: Out of a total of 6244 promoters of protein coding genes that were identified to be H3K4 trimethylated Day 8 and Day 24 mice testes in our data set, transcription profile of 3105 genes could be identified in the microarray data set. We also detected 1267 H3K4Me3 peaks of non-coding RNAs, out of which 314 were of miRNAs. The prominent members were miR34b, miR34c, miR199b, miR184, miR762 and miR475. miR34b and miR34c were especially important in that they made strong networks with mRNAs relevant to spermatogenesis. We further demonstrated that ablation of Meisetz in germ cells resulted in aberrant expression of spermatogenesis related genes, thus emphasizing the role of Meisetz dependent H3K4 trimethylation during spermatogenesis. Some of the genes analyzed such as Gpx4, Zfp35, Ccin, Theg and Zfp37, are known to be crucial to spermatid development.

Conclusion: This study establishes that H3K4 trimethylation is important in establishing spermatogenesis specific gene expression pattern by its direct effect on transcription of both coding and non-coding RNAs. Our study shows that H3K4 trimethylation is necessary for the transcription of the genes important to spermatogenesis and therefore, may play a crucial role in determining the fertility status.

Keywords: Spermatogenesis, miRNA, Transcriptome, Histone Methylation, Testis

Reproductive Imaging

I-51: Diagnosis of Uterine Anomalies by Ultrasound and MRI before Starting Treatment

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Congenital anomalies of uterine shape occur in 4% to 7% of all women. The prevalence in women with infertility and early miscarriage is up to 10%, and as high as 25% in those with midtrimester pregnancy losses. They cause a wide range of infertility, missed abortion, and pregnancy failure, so the accurate diagnosis and proper treatment is obligatory especially in such critical patients who are going to get ART. The best imaging modality are 3D vaginal sonography and MRI which according to availability and comfortability 3d sonography has salient growing recently, so the main focus of this lecture is about this modality.

Animal Biotechnology

O-1: Effects of Ethylen Glycol and Quercetin Supplementation in Cryoprotectants on Post-Thawed Kangal Dog Sperm Ultrastructure

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Background: The aim of this study was to investigate the effects of different cryoprotectants containing ethylene glycol (EG) and quercetin (Q) on post-thawed Kangal Dog sperm ultrastructure.

Material and Methods: Ejaculates were collected by digital manipulation twice a week and divided into three equal aliquots, and diluted with Tris-based extender containing EG 5% (EG group), EG 5% + Q 50 µg (EGQ50 group) and EG 5% + Q 200 µg (EGQ200 group). Subsequently, the ejaculates were cooled to 4°C at 1.5 hours, and stored in liquid nitrogen (~-196°C). Frozen straws were thawed for 25 second in a water bath. The sperm samples were prefixed at 4°C, for 2 days in Karnovsky fixative. After washing with PBS, the sperm pellet was post-fixed in 1% osmium tetroxide. They were then dehydrated in an ascending alcohol series (25 to 100 %), infiltrated with propylene oxide and embedded in araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Field Emission Scanning Electron Microscope (FESEM) was used to examine the frozen-thawed Kangal dog sperm at 20 kV.

Results: Post-thawed spermatozoa were evaluated by means of TEM in two regions: head and tail. The plasma membrane which was swollen away from the acrosome was noted in many cells in all groups. The sperm exhibited vesiculation of acrosomal and plasma membranes. Transversal sections of most sperm tails in all groups were show dilated plasma membrane. In EG group, disrupted plasma membrane was also seen. Normal plasma membranes were observed in EGQ50 and EGQ200. Normal axial 9+2 fiber pattern were found in most of the sperm tails transverse sections for all groups. But, lack of the some outer dense fibers were also observed in EG and EGQ50 groups.

Conclusion: It has been concluded that: 1. Cryopreservation caused ultrastructural changes especially to the Kangal dog sperm head and 2. neither ethylene glycol nor quercetin were able to protect sufficiently the Kangal dog sperm ultrastructure from the deleterious effects of cryopreservation in any concentrations.

Keyword: Kangal Dog Sperm, Cryopreservation, Electron Microscopy, Ethylene Glycol, Quercetin

O-2: Dopamine Intervention in Induction of Polycystic Ovary by Morphine in the VMH of Female Rat

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Background: Morphine significantly reduces the chance of pregnancy in rat and causes infertility. The occurrence of irregular menstrual cycles in humans has been reported by morphine. Also level of estradiol decreases in ovary after injection of morphine in rat. In the mechanism of morphine action, it is believed that the drug stimulates three types of opioid receptors mu, delta and kappa. Stimulation of these receptors decreases input of calcium into the cell, increase output of potassium from the cell and decrease cAMP in the cell. The release of norepinephrine is an important factor in the hypothalamus-pituitary-gonad axis. The effects of opioids on the release of dopamine in the ventromedial hypothalamus (VMH) has not been yet investigated which is our goal.

Materials and Methods: Female rats (220-300 g) kept under standard conditions. Using a stereotactic device, they were surgically coordinated: Anterior-posterior: -1/92, ventral: 9, lateral: 0.5. After a week recovery they were microinjected morphine (0.1, 0.2, 0.4 µg/rat, once intra-VMH). To investigate the dopamine intervention with the morphine sulphiride (0.1, 0.2, 0.4, µg/rat once intra-VMH) was injected intra-VMH prior to morphine to inhibit the dopamine D2 receptor. The control group received physiological saline (1 µL/rat, intra-VMH). Three days after the experiment, the uterus, the ovary and the brain samples were collected in %10 formalin and studied histopathologically using hematoxylin and eosin.

Results: The ovaries in group in which morphine was injected intra-VMH showed polycystic features as compared to the control group. With the presence of sulphiride prior to morphine intra-VMH the polycystic ovary was decreased.

Conclusion: These results indicate that morphine disrupts fertility. This effect is most probably is resolved by dopamine D2 receptor signaling blocking.

Keywords: Dopamine, Morphine, Polycystic Ovary, Ventromedial Hypothalamus, Female Rat

Embryology

O-3: Heparin-Binding Proteins, A New Predictor of The Semen Quality via Regulation Oxidant/Antioxidant Balance

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Background: Seminal plasma comprises a large collection of proteins of clinical significance which are fundamentally required to maintain the successful fertilization and the reproductive physiology of spermatozoa. Heparin binding proteins (HBPs) are created by accessory glands and secreted into the seminal fluid. HBPs are bind to the spermatozoa at the time of ejaculation, acrosome reaction, favour capacitation. HBPs acts to alter the immune system response toward the sperm. HBPs are essential components of semen, which bind to sperm lipids containing the phosphorylcholine group and regulate the fertilization process. On the other hand, Spermatozoa have large concentration of polyunsaturated fatty acids in their plasma membrane and low levels of scavenging enzymes in their cytoplasm. Therefore, it is subject to peroxidation from elevated seminal

reactive oxygen species levels. Thus, separation and identification of HBPs is of principal significance for their biophysical description and functional examination in reproductive physiological processes.

Material and Methods: Semen samples were obtained from 60 fertile and 60 asthenozoospermic infertile men of matched age. Sub-fertile male partners from couples who had consulted the infertility clinic of the Babil Hospital of Maternity (Hilla City, Iraq) from July 2015 to July 2016. Fresh semen were selected from the experimental the two groups. After liquefaction of the seminal fluid at room temperature, routine semen analyses were performed. Seminal plasma from semen was separated by centrifugation. HBPs were purified from heparin-agarose affinity column by suitable elution buffer. HBPs concentration was estimated by Lowry's method. The purified HBPs were resolved on Sodium dodecyl sulfate polyacrylamide gel electrophoresis to check the protein profile. The measured antioxidant parameters include total antioxidant status, total reactive oxygen species levels; nitric oxide levels and NO synthase activity.

Results: The mean values of HBPs concentrations in control group and in asthenospermic group were 4.31 ± 0.11 and 3.51 ± 0.17 , mg/mL, respectively. The values HBPs were significantly higher ($P < 0.01$) in control when compared to asthenospermic group. 33 kDa HBP was more intensely existed in control group, thus may indicate it's linked to fertility plausible. The biochemical parameters that measured were compared among fertile controls and infertile patients. NO synthase activity; reactive Oxygen species levels and nitric oxide levels were significantly higher in the infertile patients compared to the fertile group. Conversely, total antioxidant was significantly higher in the fertile group than the infertile patients.

Conclusion: HBPs concentrations correlated with antioxidants levels. The decrement of HBPs concentrations may be one of the central relations to cause idiopathic asthenospermia.

Keyword: Heparin-Binding Proteins, Asthenospermia, Nitric Oxide, Nitric Oxide Synthetase, Total Antioxidant

transfer for RIF patients undergoing IVF program.

Materials and Methods: This study is a randomized clinical trial, including 250 couples (PBMC-test, $n = 122$ and Control, $n = 128$) who being part of Frozen-thawed embryo transfer cycles. In tread group a blood sample is scheduled five days before frozen-thawed embryo transfer and PBMC was isolated using a separation protocol based on Ficoll. PBMC is well prepared after a culture for 48-72 h. Then, 0.4ml of cultured PBMC transferred to the patient of PBMC group, in uterus 2-3 days before embryo transfer.

Results: Clinical pregnancy rate was increase from 23.4% for control group to 34.4% for PBMC group but this different wasn't statistically significant ($P = 0.07$, chi-square). When we limited our analysis to patient with ≥ 3 RIF ($n = 138$), there were a significant difference in clinical pregnancy rate (38.6% vs 19.8%) between PBMC and control groups ($P = 0.02$, chi-square).

Conclusion: The findings of this study indicate that the use of PBMC can be an effective treatment for infertile patients with RIF.

Keywords: Peripheral Blood Mononuclear Cells, Corticotropin-Releasing Hormone, In Vitro Fertilization, Embryo Implantation, Pregnancy Rates

Female Infertility

O-4: Intrauterine Administration of Treated PBMC Prior Frozen/Thawed Embryo Transfer Improves Pregnancy Outcomes in Patients with Repeated Implantation Failures

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Background: Implantation success is mainly dependent on local immune-tolerance mechanisms involving a spectrum of cytokines, interleukines and growth factors. The aim of this study was to evaluate clinically the effectiveness of intrauterine administration of treated PBMC prior frozen/thawed embryo

Andrology

P-1: Oxidative Stress Induced by Cadmium Chloride in Human Sperm Viability and Motility-Protective of Silymarin

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Background: Cadmium, as an environmental pollutant, exerts its destructive effects on male reproductive system and sperm by inducing oxidative stress. Silymarin, an effective substance extracted from silybum marianum, is a potent antioxidant which inhibits oxidative stress. Therefore, the use of antioxidants, especially natural antioxidants, with the aim of removing free radicals, as well as strengthening the antioxidant defense system can be considered as an appropriate strategy to minimize of oxidative stress-effects to prevent infertility. The aim of this study was to investigate if silymarin can protect the harmful effects of cadmium chloride on human sperm motility, viability, total antioxidant capacity and lipid peroxidation.

Materials and Methods: In this study, high quality spermatozoa were used. The samples were washed by human tubal fluid containing bovine serum albumin. The sperm suspensions were divided into 5 groups (2×10^7 spermatozoa per group). 1. Spermatozoa at 0 hour 2. Spermatozoa incubated for 180 minutes (control) 3. Spermatozoa treated with cadmium chloride (20mM) for 180 minutes 4. Spermatozoa treated with silymerin (2mM) for 180 minutes 5. Spermatozoa treated with silymarin+cadmium chloride for 3 hours. Sperm motility was performed according to World Health Organization (WHO) guidelines and eosin nigrosin staining, while malondialdehyde (MAD) and ferric reducing antioxidant power (FRAP) were assessed to investigate lipid peroxidation and total antioxidant capacity respectively. The results were analyzed using one-way ANOVA and $P < 0.05$ was considered significant.

Results: Cadmium chloride reduced a significant ($P < 0.000$) in percentage of motility, viability and total antioxidant capacity, while increased ($P < 0.001$) the amount of MAD compared to the control group. In the silymerin+cadmium chloride group, silymarin could significantly ($P < 0.001$) reverses the toxic effect of cadmium chloride on these parameters, when compared to the cadmium chloride group. The application of silymarin alone significantly ($P < 0.001$) increased motility, viability, FRAP and decreased the amount of MAD as compared to the control group.

Conclusion: Cadmium chloride by inducing oxidative stress exerts toxic effects on motility, viability, FRAP and MAD and silymarin as a potent antioxidant compensate the adverse effects of cadmium chloride.

Keywords: Cadmium Chloride, Silymerin, Motility, Variability, Human Sperm

P-2: Evaluation of Sperm DNA Damage in Male Rats Induced by Varicocele

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Background: Varicocele is an abnormal inflation of the pampiniform venous in the scrotum that is considered as one of the main causes of infertility in men, and are associated with increased levels of oxidative stress and apoptosis in the testis. Therefore, this study aimed to assess sperm DNA damage in male rats induced with varicocele experimentally.

Materials and Methods: In the present study, 30 adult male Wistar rats were divided into three groups, I: varicocele-induced II: sham and III: control. After two months of varicocele induction, rats were sacrificed and epididymides were dissected. Then, sperm parameters and sperm DNA damage were assessed by WHO protocol, and acridin orange test. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Tukey). Collected data were presented as mean \pm standard error of mean (SEM) and $P < 0.05$ was considered to be significant.

Results: The result of the current study showed that mean of sperm parameters such as sperm concentration, motility and morphology were significantly lower in varicocele induction group compared to control and sham groups ($P < 0.001$), while percentage of sperm DNA damage were significantly higher in varicocele induction group compared to control and shame groups ($P < 0.05$).

Conclusion: In the varicocele status due to the high testicular temperature, and consequently increase of oxidative stress level, percentage of DNA damage in sperm have increased. Therefore, antioxidant therapy can be an efficient approach for reduction of oxidative stress and DNA damage.

Keywords: Varicocele, Sperm DNA Damage, Sperm Parameters, Oxidative Stress, Acridin Orange Test

P-3: Effect of Pre-Incubation Sperm Time from Testicular Sperm Extraction (TESE) on Fertilization Rate and Embryo Quality

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Background: Non-obstructive azoospermia (NOA) is a condition with the severely impaired production of sperm or no absence sperm production in ejaculated semen. Approximately 60% of male factor infertility is non-obstructive azoospermia. Testicular sperm extraction (TESE) is commonly used to obtain sperm for intracytoplasmic sperm injection (ICSI) in men with non-obstructive azoospermia. Because sperm has a finite lifespan, current protocols for harvesting sperm be performed on the same day as the retrieval of the oocytes from the partner. The aim of this study was to evaluate the effect of pre-incubation sperm time from TESE on fertilization rate and embryo quality.

Materials and Methods: Testicular specimens were retrieved patients with nonobstructive azoospermia who underwent for ICSI at the Infertility Treatment Center Of Besat Hospital, Sanandaj, Iran. The biopsy samples are placed in a petri dish and using fine needles, the samples are rinsed with culture media divided into small pieces. Small pieces were subdivided into 2

groups prior to ICSI as Group I - sample pieces not incubated, Group II - sample pieces incubated 2- to 3 - hours in culture media. Then, the mean of fertilization rate and embryo quality were compared between two groups.

Results: The current study showed that the sperm concentration of TESE was improved after preincubation ($P<0.05$); and also the mean percentage of fertilization rate and embryo quality were significantly higher in the group II than in the group I ($P<0.05$).

Conclusion: It was concluded that a delay of 2-3 hours prior to ICSI has a beneficial impact on fertilization and embryo quality.

Keywords: NOA, TESE, ICSI, Pre-Incubation

P-4: Changes in Serum Testosterone and Alkaline Phosphatase Activity in Testis Tissue after Administration of Berberine in Experimental Varicocele

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Background: Varicocele (VCL) is one of the greatest andrological pathologies since 35 to 40% of male subfertility is due to varicocele. Serum levels of testosterone in varicocele significantly reduced. In addition, alkaline phosphatase activity in testis tissue in varicocele increases. Present study was done in order to evaluate the changes in serum level of testosterone and evaluate alkaline phosphatase activity in testis tissue after administration of berberine in experimental varicocele.

Materials and Methods: Thirty mature male Wistar rats were randomly divided into control (NO: 6 rats), control-sham (NO: 6 rats) and experimental groups (NO: 18 rats). The animals in experimental groups were undergone experimental varicocele and simple laparotomy was conducted in control-sham group. The experimental group subdivided into: Non-treated VCL-induced, 50 mg/kg and 100 mg/kg berberine-treated groups. The serum levels of testosterone and alkaline phosphatase activity in testis tissue were assessed.

Results: Observations revealed a significant ($P<0.05$) reduction in serum levels of testosterone and a significant ($P<0.05$) increase in alkaline phosphatase activity in testis tissue in non-treated VCL-induced animals versus control and control-sham groups. No significant changes were found between control and control-sham groups. Meanwhile, the berberine-treated animals (especially 100 mg/kg) exhibited a remarkable ($P<0.05$) enhancement in Serum levels of testosterone and a significant ($P<0.05$) reduction in alkaline phosphatase activity in testis tissue.

Conclusion: According to our finding, Berberin by increasing in serum levels of testosterone increases the Testicular endocrine capacity and protects Leydig cell against Inflammatory and oxidant injury varicocele. In addition, Berberin by decreasing in alkaline phosphatase activity in testis tissue, reduces inflammatory damage of varicocele.

Keywords: Varicocele, Berberine, Testosterone, Alkaline Phosphatase

P-5: Experimental Varicocele-Induced Inflammation Re-

sults in Spermatogonial Stem Cells (SSCs) Apoptosis

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Background: Varicocele (VCL), as one of main reasons of male infertility, results in spermatogenesis arrest. Previous studies have been illustrated that, the VCL negatively affects the testicular antioxidant status and endocrine potential, and consequently it results in a remarkable apoptosis in germ cells. Apart from the effect of oxidative stress against germ cells, the mechanism by which the VCL-induced inflammation is able to potentially end with apoptosis has not been reported. Therefore, current study was conducted to assess the cross-link between post VCL-induced inflammation and spermatogonial stem cells (SSCs) apoptosis in a time dependent study, using animal model.

Materials and Methods: 18 mature male Wistar rats were divided into 3 groups, including control (with no surgical intervention), control-sham (with simple laparotomy) and VCL-induced animals. The animals were euthanized following 2 months and the testicles were dissected out. The testicular nitric oxide level as well as the numbers of iNOS⁺ cells/mm² of tissue were analyzed. The testicular interleukin-6 (IL-6) was assessed by western blotting. The SOX-2 was considered as biomarker of the SSCs and the SOX-2⁺ cells distribution/mm² as well as protein level of SOX-2 were investigated using immunohistochemistry and western blotting techniques. The numbers of apoptotic SSCs/mm² were counted. Finally, the findings were analyzed by One-way ANOVA, and the correlation between SSCs number/mm² of tissue and inflammatory mediators was estimated.

Results: Observations revealed that, the VCL significantly ($P<0.05$) increased the testicular level of nitric oxide and remarkably ($P<0.05$) elevated the iNOS⁺ cells/mm² of tissue versus control and control-sham animals. Moreover, the animals in VCL group exhibited increased expression of IL-6 compared to control and control-sham group. Finally, the animals in VCL group represented decreased expression of SOX-2, and at the same time the number of SOX-2⁺ cells were decreased in seminiferous tubules. Finally, the apoptotic SSCs distribution was increased in VCL group. Moreover, the positive correlation between apoptosis and each inflammatory mediator (especially IL-6 and apoptotic cells) as well as negative correlation between inflammatory mediators and SOX-2⁺ cells were demonstrated.

Conclusion: Our data showed that, not only the VCL-induced oxidative stress but also the VCL-induced inflammatory mediators are able to induce apoptosis in SSCs and adversely affect the SSCs repopulation.

Keywords: Varicocele, Apoptosis, SSCs, Inflammation, Rat

P-6: Protective Effect of Silymarin on Cadmium-Induced Apoptosis in Human Sperm

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Background: Infertility could be one of tragic realities in industrial areas. Approximately 40-50% of infertility is related to men. In industrial cities, the incidence of infertility may be due to environmental pollutants and heavy metals. Cadmium is a heavy metal and environmental pollutant that induces apoptosis in different types of tissues and cells. Cadmium is also able to exert its adverse effects on male reproductive system and sperm through apoptosis. Several lines of studies suggest that cadmium can induce apoptosis in the cells via caspase-dependent and independent pathways. Apoptosis, programmed cell death, is a form of cell death that occurs in all multicellular organisms for removing damaged cells. DNA fragmentation, cell shrinkage, the decrease of mitochondrial membrane potential and the activation of caspases are some features of apoptosis. The aim of this study was to investigate mechanism by which apoptosis is induced in human spermatozoa treated with cadmium chloride and if silymarin, as a potent antioxidant, is able to prevent the toxic effects of cadmium chloride.

Materials and Methods: Human ejaculated spermatozoa were divided into five groups: 1. spermatozoa at 0 hour, 2. spermatozoa at 180 minutes (control), 3. spermatozoa treated with cadmium chloride (20 μ M) for 180 minutes, 4. spermatozoa treated with silymarin (2 μ M) and cadmium chloride (20 μ M) for 180 minutes, 5. spermatozoa treated with silymarin (2 μ M) for 180 minutes. DNA integrity was performed by sperm chromatin dispersion test (SCD). Nucleus diameter was evaluated by diff-quick staining, whereas flow cytometry was used to assess sperm size. Rhodamine 123 staining and the application of cleaved caspase-3 antibody were used to study mitochondrial membrane potential and the activation of caspase-3, respectively. One-way analysis of variance (ANOVA) followed by the Tukey's test was used to assess the statistical significance of the data and $P < 0.05$ was considered significant.

Results: Cadmium chloride significantly decreased DNA integrity, nucleus diameter, sperm size and mitochondrial membrane potential compared to the control group. Immunocytochemistry study showed no intense immunoreactivity against the antibody in the cadmium chloride group. In the silymarin + cadmium chloride group, silymarin could compensate the adverse effects of cadmium chloride on DNA integrity, nucleus diameter and mitochondrial membrane potential.

Conclusion: Cadmium chloride exerts apoptosis in human spermatozoa through a caspase-independent manner and silymarin compensate the toxic effects of cadmium chloride.

Keywords: Human Sperm, Silymarin, Cadmium Chloride, Apoptosis

P-7: Effect of Phoenix Dactylifera Pollen on Expression of Nrf2, SOD2, GPX4 and CAT Genes, and Sperm Parameters in Infertile men

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Background: Oxidative stress is the result of an imbalance between the production and scavenging of reactive oxygen species (ROS), and has been described as a major cause of male infertility in 30-40% of cases overall. The positive impact of Phoenix Dactylifera Pollen (Date Palm Pollen, DPP) consumptions on the improvement of sperm parameters of animal models has been well documented. However, there is little scientific evidence in support of the possible molecular mechanisms involved in its efficacy on human fertility.

Materials and Methods: This study aimed to evaluate the effect of DPP consumptions on the sperm parameters, ROS levels, and expression pattern of antioxidant genes and activity of antioxidant enzymes of infertile men. A total of 60 patients diagnosed with male infertility and 20 normospermic fertile men were recruited into the study. Before and after treatment of patients with DPP, 400 mg/kg in gelatinous capsules every day for 30 consecutive days, semen samples were taken. Semen analysis was performed using Computer-assisted sperm analysis (CASA). Level of free 8-Isoprostane and activity of superoxide dismutase, glutathione peroxidase and catalase were assessed by commercially available enzyme immunoassay method. Quantitative real-time PCR was used to evaluate of mRNA expression levels of Nrf2, SOD2, GPX4 and CAT genes.

Results: Our results demonstrated that DPP consumption lead to significant improvement of semen parameters including count, motility, morphology of sperms and a significant reduction of 8-Isoprostane levels ($P < 0.05$). After treatment of patients, the mRNA expression level of Nrf2, SOD2, GPX4 and CAT genes and the enzymatic activity of superoxide dismutase, glutathione peroxidase and catalase significantly increased ($P < 0.05$). Moreover, we found that the increased expression of all antioxidant genes and enzymes significantly correlated with improvement of semen parameters including count, motility and morphology of sperm ($P < 0.05$). A significant correlation between alteration of SOD2 gene expression and superoxide dismutase activity, GPX4 and glutathione peroxidase, CAT and catalase were also observed ($P < 0.05$).

Conclusion: Our findings provide additional evidence to support the efficacy of DPP on the enhancement of male fertility. It seems that treatment with DPP can ameliorate the deleterious effects of ROS, at least in part, by activating antioxidant systems of sperm and inducing antioxidant genes expressions.

Keywords: Male Infertility, Phoenix Dactylifera Pollen, SOD2, GPX4, CAT

P-8: Can Reacted Acrosomes Be Increased in Ejaculated Human Spermatozoa with Bacterial Infection?

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Background: One of the essential events during fertilization process is the normal acrosome reaction that several factors may induce the premature acrosome reaction. Also, bacterial semen infection is an important problem in the pathophysiology of human subfertility/infertility. Therefore, this study was

planned to assay whether bacteriospermia could increase premature reacted acrosome rate of semen samples from infertile men.

Materials and Methods: In this research, 70 semen samples from infertile men undergoing assisted reproductive treatment were collected and microbiological analysis of semen samples was performed within 1 hour after ejaculation from 2017 to 2018. The acrosome reaction and viability of spermatozoa were assessed by the triple staining technique. The percentage of spermatozoa with reacted acrosome was evaluated for each sample, and analyzed using SPSS software.

Results: Overall, 40.4% of studied samples were infected with bacteriospermia (such as *E. Coli*, *Staphylococcus saprophyticus*, etc.). The results show that the semen samples infected with bacteria had significantly more spermatozoa with reacted acrosomes and dead spermatozoa ($P < 0.05$) compared to samples without bacterial infections.

Conclusion: It is concluded from this study that one of the most important sperm function such as acrosome reaction are impaired in men with bacterial infection.

Keywords: Bacteriospermia, Acrosome Reaction, Viability, Male Infertility

P-9: Experimental Study of Sperm Separation for *In Vitro* Fertilization Applications Using Inherent Instincts of The Fluid and Sperms Motility

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Background: Nowadays, applications and performance of microfluidic chips in medicine and engineering is outstanding. In cases such as cancer, there have been many achievements in diagnosis, treatment, purification and drug delivery, by using variable procedures on a microfluidic chip. In this research, the semen sample would be purified from other debris and the final sample would be manipulated in order to auto select the high quality sperms. The unique design of this microchip is based on the inertial-force-induced separation. The flow Reynolds number is the ratio of inertial forces to viscous forces. Another dimensionless number is particle Reynolds number; criteria that states the dominance of rather inertial or viscous forces, that can be expressed by the size ratio of the particles to the channel length scales (a/D_h).

Materials and Methods: The first section of this device is the inertial separation section, which allows the smaller particles (sperms) to move along the streamlines, while the other particles (WBCs) move straight forward due to their higher diameter and density. As the smaller particles are going through the side channels, there are some microarrays in order to filter the sample from probable debris or impurities. In this section, the purity of the final sample will be guaranteed by using micro-pillars. In the last section of the chip, as the velocity of the flow will decrease dramatically, there are two side channels with divergence angle of 45° in order to capture the left or right-swimmer sperms. As it has been addressed in recent researches, sperms with the ability to swim to the left or right, are capable of having higher HDS (High DNA Stainability) and also less

DFI (DNA Fragmentation Index).

Results: By using a combination of hydrodynamic technique and microarrays, a new method of sperm purification has been evaluated which is label free and also leads us to a non-intrusive and non-invasive separation procedure. This microchip provides a very faster process through sperm purification, also benefits a pre-collection of high quality sperms for *in vitro* fertilization (IVF) procedure.

Conclusion: In this research we designed a microchip which is able to purify the semen sample from other debris, also auto select the high quality sperms for being used in the IVF procedure. This microchip would be a suitable filed for other sections (magnetic field) in order to do the sex-selection as well. Also, this microchip could be commercialized as a home use device in order to do a pre-assessment on semen sample at home for further information about the quality of the semen sample.

Keywords: Microchip, Sperm Separation, Motility, Inertial Forces, High Quality Sperms

P-10: Evaluation of Sperm Chromatin Quality and Apoptosis Rate in Opium Addicted Men

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Background: Oxidative stress generated by opioids can be detrimental to human sperm. Morphine, as the main component of opium latex increases the production of free radicals and may affect sperm DNA integrity and chromatin quality. Background: To investigate the chromatin quality and DNA integrity of sperm in men with the history of morphine abuse.

Materials and Methods: A total of 30 opium-addicted men and 30 healthy controls were recruited for the study. The case subjects were categorized into three different groups: men who abused opium (group 1); men with a history of addiction to both opium and cigarette (group 2); and men addicted to both opium and its derivatives (opium extract) with a history of cigarette smoking (group 3). Semen collection was in accordance with WHO criteria (2010). To determine the sperm chromatin abnormalities, aniline blue (AB) and toluidine blue (TB) staining methods were applied. The percentage of apoptotic spermatozoa was evaluated by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. The data were statistically analyzed by one-way analysis of variance (ANOVA), student's t test and Mann-Whitney U test. $P < 0.05$ was considered significant.

Results: There was a significant increase in the rate of aniline blue-positive spermatozoa as well toluidine blue-positive spermatozoa in the opium addicts compared to the controls ($P < 0.05$). Moreover, case group had a higher percentage of spermatozoa with apoptosis (TUNEL positive) compared to the controls significantly ($P < 0.05$). However, there were no significant differences between case subgroups with regard to aniline blue and toluidine blue staining in addition to TUNEL assay ($P > 0.05$).

Conclusion: Morphine abuse can adversely affect the sperm chromatin and DNA integrity regardless of the kind of opiates.

Keywords: Opium Addiction, Morphine, Sperm Chromatin , Oxidative Stress, Apoptosis

P-11: Effect of RNA Later on RNA Integrity in The Snap Freezing of Testis Tissues

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Background: Human tissues biobanking encompasses a wide range of activities and study designs and also has a critical role for application of new technologies to the discovery of molecular patterns of disease and novel biomarkers into clinical trials. Testis tissue plays a dominant role in diagnostic and treatment azoospermia patients. The purpose of this study was to assess the impact of freezing on RNA integrity in tissue specimens and also comparison between immersion in liquid nitrogen (unfixed) and immersed (fixed) in a commercial RNA-stabilizing buffer (RNAlater).

Materials and Methods: In this study, 30 samples of infertile men with azoospermia were evaluated. Collecting the samples was performed during the surgical resection at Royan Infertility Clinic. Following that, the 15 tissues were frozen directly by immersion in liquid nitrogen (-196°C) and the 15 testis samples were preserved in RNA later and stored in -80 (fixed). RNA integrity was analyzed by agarose gel electrophoresis. In addition, the RNA integrity number (RIN) was measured by the Agilent 2100 Bioanalyzer.

Results: The portion of 28S/18S in fixed samples was dramatically higher than in unfixed samples. In unfixed samples degradation of RNA increased. In addition RIN in unfixed samples significantly decreased than in fixed samples.

Conclusion: Our study shows that the immersion of the tissues in RNAlater efficiently prevented RNA degradation. Thus, degradation of RNA in unfixed samples occurs. Already decrease in RNA quality may result in significantly decrease in RIN values in testis tissue samples. According to the RIN results, we recommend that the preservation of testis tissues with RNAlater is better than frozen without RNAlater for studies requiring RNA of high quality. Our study shows that the immersion of the tissues in RNAlater efficiently prevented RNA degradation. Thus, degradation of RNA in unfixed samples occurs. Already decrease in RNA quality may result in significantly decrease in RIN values in testis tissue samples. According to the RIN results, we recommend that the preservation of testis tissues with RNAlater is better than frozen without RNAlater for studies requiring RNA of high quality. Our study shows that the immersion of the tissues in RNAlater efficiently prevented RNA degradation. Thus, degradation of RNA in unfixed

samples occurs. Already decrease in RNA quality may result in significantly decrease in RIN values in testis tissue samples. According to the RIN results, we recommend that the preservation of testis tissues with RNAlater is better than frozen without RNAlater for studies requiring RNA of high quality.

Keywords: Testis Tissue, RNA Integrity Number, RNAlater

P-12: Gene Expression Comparison of The Nox5 and Sperm Parameters in Oligospermic Men Versus Normal Men

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Background: Infertility is a global issue which has affected 15-20% of couples. Within the past two decades, understanding of reproduction system and its related factors has grown dramatically, especially the role of male factor in infertility. Previous research has shown that 20% of infertilities are caused by male infertility. Oxidative stress which leads to the production of active oxygen types (ROS) is recognized as a key factor involved in male infertility. However, its physiological values are estimated by the enzymes in NADpH family. In the case of disrupted spermatogenesis and maintenance of extra cytoplasm, there is an increase of ROS in unnatural sperm. Moreover, there is research-based evidence that sperm parameters also affect infertility.

Materials and Methods: In this research, the sample consisted of 50 healthy and 50 oligospermic male subjects. A semen sample was taken from both groups to analyze sperm parameters as well as mRNA expression in Nox5 gene.

Results: Sperm parameters including sperm count, volume and morphology in the seminal fluid of the control group enjoyed a better and more proper state than the oligospermic group. Analysis of Nox5 expression showed to be increased in the oligospermic group as compared to the healthy group.

Conclusion: The present findings confirmed the effect of oxidative stress as involved in male infertility. Therefore, a proper therapeutic approach can hope to reduce ROS level with the help of antioxidants and improve sperm quality. It can also reduce the expression of oxidant genes such as Nox5 and thus increase male fertility.

Keywords: Infertility, ROS, NOX5

P-13: Evaluation of The Effects of N-Acetyl-Cysteine on Sperm Quality, Chromatin Integrity and Levels of The Antioxidant Enzyme in Infertile Men

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Background: To evaluate effects of supplementation with N-acetyl-cysteine (NAC) on the sperm parameters and biochemical factors in terato-asthenozoospermia production of reactive oxygen species (ROS) is a normal physiologic event in various organs, but excessive production of ROS can disrupt sperm function, lipid peroxidation, loss of motility and sperm DNA damage.

Materials and Methods: The study was carried out in Unit of Infertility Research Center of the ACECR (Qom, Iran). This study was performed on 50 infertile men with terato-asthenozoospermia. The patients were given NAC (600 mg/d orally) for 3 months; the control group before received NAC. The semen analysis was performed according to WHO (2010), protamine content [chromomycin A3 (CMA3)], DNA integrity [terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)] assessed. TAC (total antioxidant capacity) and MDA (Malondialdehyde) as stress oxidative markers were determined with by ELIZA kit.

Results: Percentage of abnormal semen parameters, protamine deficiency, DNA fragmentation were significantly decreased in patients was given NAC. At the end of study, the total sperm count, sperm motility and normal morphology in patients were significantly higher than before NAC treatment. TAC was significantly increased but MDA lower than NAC receiving, Data showed a negative correlation between TAC and MDA levels in infertile men.

Conclusion: According to the results, medical therapy of terato-asthenozoospermia with NAC supplement could improve quality of semen parameters and chromatin integrity. High degree of correlation between sperm parameters and antioxidant enzymes suggests reactive oxygen species resulting in reduced sperm quality and levels of antioxidant enzyme.

Keywords: N-acetyl-cysteine, Reactive Oxygen Species, Terato-asthenozoospermia, Sperm Parameters, Antioxidant Enzyme

P-14: Efficient Concentration of Zinc *In Vitro* Improves Human Sperm Motility

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Background: Zinc ion in human body is known as a micro-nutrient and also cofactor for more than 300 enzymes so it is

essential for large number of cell functions. High concentration of zinc could be found in prostate and parts of the eye. Zinc concentration is about 30 times higher in seminal plasma than in blood of men. In some studies, sperm motility improvement in asthenozoospermia and even oligozoospermia, has been reported 3 months after oral zinc supplements. *In vitro* studies on animal and human semen samples showed that adding concentrations lower than 100 μ M of zinc, depends on added concentrations and animal species, caused sperm motility improvement. A Recent study showed that seminal zinc concentration was higher in asthenozoospermia than in normozoospermia cases, they demonstrated that high zinc levels could corrupt sperm motility. The aim of this study was to investigate the changes of sperm motility after zinc treatment of semen in asthenozoospermia men referred to Royan institute.

Materials and Methods: Semen samples were collected from 20 normozoospermic and 39 asthenozoospermic men. After simple wash, sperm was exposed to different concentrations of zinc sulfate (0.1, 1, 10, 100 μ M) for three hours and also there was a zinc free group (0 μ M). Before that seminal plasma Zinc level was determined by spectrophotometry. Sperm motility was evaluated before and after zinc treatment by CASA system. A Randomized Block Design was performed for Data analyzing.

Results: The significant increase was seen in total and progressive sperm motility of normozoospermic group after 10 μ M zinc treatment. However, in asthenozoospermic samples only with semen zinc concentrations less than 140 μ g/ml, an improvement in sperm motility was seen in this concentration.

Conclusion: Zinc in appropriate concentration could have a beneficial effect on sperm motility in normozoospermic men. Zinc treatment in semen of asthenozoospermic men with high seminal plasma zinc concentration results no improvement on sperm motility.

Keywords: Seminal Zinc, Sperm Motility, Asthenozoospermic Men

P-15: Survey on Effect Saffron as Antioxidant on Sperm Parameters in The Men by Normal Spermatogenesis after Freezing and Thawing Process

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Background: Sperm freezing method is used frequently in assisted reproductive techniques, on the other hand in different studies negative effect of freezing have been shown on different sperm parameters. The aim of this study was to determine the effect of saffron extract as an antioxidant, on the different sperm parameters in men with normal spermatogenesis after freezing-thawing process.

Materials and Methods: In this case-control study, collecting of samples was done in 2015 year from the Infertility Treatment Center, ACECR Branch of Qazvin, Qazvin, Iran. These men had normal spermatogenesis and their spouse had infertility problem. Semen samples were divided in two groups, control

without saffron extract, and case with 50 mg/ml saffron extract. Then, samples freezed with snap freezing method. After two weeks, they were thawed and different sperm parameters were assessed. Data were analyzed by two-tail T test.

Results: Our results showed, mean percent of viability (72 ± 0.99), motility (87 ± 0.43), and the number of sperm cells (62.5 ± 3.8) in treaded group was elevated significantly ($P < 0.01$) compared to the control group (46.6 ± 1.1), (62.3 ± 0.33), and (45 ± 4.3) respectively. However, the morphology did not show any significant difference ($P > 0.05$).

Conclusion: Our results showed that possibly antioxidant agents of saffron extract could scavenge free radicals and thus, optimize different sperm parameters (viability, motility, and number) after freezing and thawing.

Keywords: Saffron Extract, Freezing, Thawing, Sperm Parameters

P-16: Insight to Testiculoprotective Effect of Royal Jelly in Experimentally-Induced Scrotal Heat Stress in Rats

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Background: Testicular heat stress (HS) even for a single short period can induce destruction in testicular tissue and spermatogenesis disturbances. Royal jelly (RJ) has several pharmacological activities such as antioxidant, anti-inflammatory, anti-tumor, antimicrobial and immune-modulatory functions. The current study aimed to elucidate the protective effects of RJ on HS-induced testicular damages in rats.

Materials and Methods: In this experimental study, 48 healthy Wistar male rats were randomly divided into 8 groups ($n=6$); Group 1 received normal saline, group 2 received RJ (100 mg/kg/day; PO), groups 3, 4 and 5 were heat-stressed (43, 39 and 37 °C for 20 min/day, respectively) and groups 6, 7 and 8 were heat-stressed receiving RJ (43, 39 and 37 °C for 20 min/day, respectively plus RJ at a dose of 100 mg/kg/day; PO). The HS was induced through immersion of rats scrotums in a water bath. At the end of 48 days, immunohistochemical (PCNA and TUNEL) examinations, histopathological analyses and oxidant/antioxidant status evaluation as well as p53, bcl-2 and caspase-3 mRNA expressions determination in testicular tissue were carried out.

Results: Co-administration of RJ remarkably restored HS-evoked histopathological alterations in rats testicular tissue. Following RJ co-treatment, tissue malondialdehyde (MDA) level was decreased; while tissue total antioxidant capacity and catalase levels were significantly increased compared to HS-exposed groups. Moreover, HS induced significant down-regulation of bcl-2 and up-regulation of p53 and caspase-3 in a temperature-dependent manner versus control group. However, RJ co-treatment led to notable diminishing of p53 and caspase-3 expressions and bcl-2 expression increase. The HS resulted in significant testicular germ cells apoptosis elevations in a temperature-dependent manner and reduced PCNA expression in spermatogenic cells. Interestingly, RJ improved HS-induced above-mentioned changes through TUNEL positive index reduction and PCNA index increase.

Conclusion: Our data suggest that RJ can effectively ameliorate experimental HS-induced testiculopathies in rats through testicular antioxidant defense system restoration and germ cells apoptosis regulation.

Keywords: Heat Stress, Royal Jelly, Oxidative Stress, Testis, Apoptosis

P-17: Does Insulin, as Choice Agent, Recover The Diabetes-Induced Germ Cell Cycle Arrest during Spermatogenesis; An Experimental Trial

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Background: Diabetes is majorly sub categorized into: diabetes mellitus or type I (DM) and type II. The diabetes mellitus is associated with insulin disarrangement, and is phenotypically manifested with hyperglycemia. Thus, in the case of DM, the insulin-therapy has been accepted as a routin medication with high efficiency on cellular and tissue metabolism. It has been illustrated that, the cyclins (especially cyclin D1) and the kinases (especially CDK-4) in association with kinase inhibitor p21 are able to fairly maintain and/or inhibit the cell cycle arrest during mitosis and meiosis of germ cells. Considering the potential effect of diabetes-induced oxidative stress and metabolic failure against DNA integrity, the present study was aimed to uncover the cross-link between two mentioned majorities with cell cycle machinery via focusing on the role of cyclin D1, CDK-4 and the inhibitor p21 (as kinase inhibitor) and p53 as mediator element between p21-induced apoptosis.

Materials and Methods: Thirty mature Wistar rats were divided into 3 groups: Control, DM-induced (by single injection of 50 mg/kg of streptozotocin, intraperitonealy) and insulin-treated DM-induced (DMI). After 56 days, the histological alterations, the tubular differentiation (TDI), and spermiogenesis (SPI) indices were assessed by H&E staining. Moreover, the TUNEL staining was performed to estimate the apoptois index. The expressions of p53, p21, cyclin-D1 and cdk-4 were evaluated using RT-PCR and immunohistochemistry staining (IHC) techniques, respectively. Finally, to reduce the bias problems/errors the positive reactions of TUNEL and IHC staining techniques were re-evaluated by pixel based frequency analyzes and the positive cells distribution percentage per mm² of testicular tissue were counted and compared between groups.

Results: The animals in DM-sole group exhibited diminished TDI and SPI ratios by representing the enhanced percentage of tubules with negative germ cell differentiation as well as spermiogenesis. However, the insulin remarkably ($P < 0.05$) improved TDI and SPI ratios. The DMI animals represented no remarkable improvement in cdk-4 expression, while they exhibited enhanced cyclin D1 mRNA and protein contents. No statistical changes were revealed in mRNA level of p21 in DMI group, whereas significantly lower p21+ cells were revealed in the same group versus DM-sole animals. Finally, the animals in the DMI group showed diminished expression of p53 as well as apoptotic cellularity compared to DM-sole group.

Conclusion: To wrap it up, the present study illustrated that, the insulin-therapy was remarkably ameliorated DNA damage and p53 overexpression in testicular tissue. Meanwhile simultaneously the insulin could not potentially rainforce the cell

cycle machinery. Because, the expression of cdk-4 did not alter significantly after insulin administration. As a logic theory, despite of insulin-induced reduction in p21 protein content, the appropriate cdk-4 expression was not found in the germ line, leading to lame cell cycle progression. Taking together, it could be concluded that the insulin, potentially inhibits the apoptosis ratio, while it is not able to directly/effectively impact the cell cycle machinery, at least in the case of cyclin D1-cdk-4-related pathway. More analyses are needed to find the other possible effects of insulin on cell cycle progression in diabetic cases.

Keywords: Diabetes Mellitus, Insulin, Germ Cell, Cell Arrest

P-18: Experimental Varicocele Negatively Affects The Spermatogonial Stem Cells Self-Renewal at VI and XII Stages of Spermatogenesis by Suppressing Nanog-Related Mechanism; Cross-Link with Niche Factors and Cell Cycle Regulators

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Background: The spermatogonial stem cells division is partially regulated by cyclin D1 and cyclin dependent kinase (CDK-4) interaction. Moreover, the glial cell line-derived neurotrophic factor (GDNF), plays a critical role in regulating the SSCs self-renewal, especially at I-VI stages of spermatogenesis. In line, the interaction of GDNF with its receptors c-RET and Gfra1, finally ends to Cyclin D1 and CDK-4 interaction. The P21, as CDK-inhibitor protein, negatively influences the SSCs division via interacting with the cyclinD1/CDK4 complex and/or through suppressing the Nanog expression, as key regulator of SSCs self-renewal at stage XII. The present study was conducted to illustrate the cross-link between cell cycle regulators and Sertoli cells-related niche elements as well as their interaction in unique network with SSCs proliferation in experimental varicocele (VCL) condition.

Materials and Methods: 12 mature male Wistar rats were divided into two groups; control-sham group (with simple laparotomy) and 2 months VCL-induced group. The mRNA and protein levels of Cycin D1, CDK4 and P21 as well as stem cell markers (GDNF, Gfra1, c-Ret, Nanog) were analyzed by RT-PCR and western blotting techniques, respectively. the immunohistochemistry staining was done to analyze the protein expression at I-VI and XII stages of seminiferous tubules. Finally, the correlations between the data of GDNF, Gfra1, c-Ret, Cyclin D1, CDK-4 and p21 with Nanog expression was analyzed.

Results: The animals in VCL-induced group exhibited diminished GDNF, Gfra1, c-RET, Nanog, Cyclin D1 and CDK-4, and represented enhanced p21 expressions versus control group. The seminiferous tubules at I-VI and XII stages exhibited enhanced expression of p21+ cells, and represented a significant reduction in GDNF+, Gfra1+, c-RET+, Nanog+, Cyclin D1+ and CDK-4+ and Nanog+ cell population. Finally, the statistical analyses revealed a positive correlation between GDNF, Gfra1, c-Ret and Cyclin D1, CDK-4 (at stages of I-VI). Moreover, the Nanog expression was negatively correlated with p21 expres-

sion (at stage of XII).

Conclusion: The VCL pathologically affects the Nanog-dependent cell proliferation via suppressing the Sertoli cells-maintained niche factors and suppressing the cell cycle machinery. Thereafter, suppressed Nanog expression significantly down-regulates the SSCs self-renewal, leading to arrested spermatogenesis.

Keywords: Varicocele, Cell Cycle, Spermatogonial Stem Cells, Sertoli Cell Niche, Rat

P-19: Conflicting Effects of Methylphenidate on Testicular Function following Dose Dependent Administration of Nicotine in Adult Rats: Epididymal Sperm Count

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Background: Testicular tissue is one of the most sensitive tissues that could be affected from environmental risk factors. Structural and functional alterations of testicular germ cells can influence the spermatogenesis process. Methylphenidate (MPH) is one of the most common medications used for treatment of ADHD syndrome that used for maintaining alertness and improving of attention which, may lead to increase of the risk of substance abuse in some cases. Nicotine (NCT) is toxic substance. It has been reported various alterations in testicular structure and function induced by nicotine. The aim of this study was to evaluate the coadministration of MPH and NCT on the sperm population in adult rats.

Materials and Methods: 30 adult rats were divided into three Control, Low NCT+MPH and High NCT+MPH groups. Low dose and high dose of nicotine (2 and 4 mg/kg/day i.p.) was administered for 8 weeks. Methylphenidate (10 mg/kg/day i.p.) was administered to NCT treated rats in whole of study period. Epididymal sperm was collected for sperm count analysis.

Results: The mean of epididymal sperm count was reduced in both treated groups in comparison to control animals. The most reduction in sperm population was observed in Low NCT+MPH group. The administration of MPH to high dose NCT treated animals was led to increase of sperm population compared to other treated group.

Conclusion: It is believed that, the MPH works through stimulation of dopamine transporter and D2 receptors. Nicotine is a potent parasympathomimetic stimulant. NCT indirectly promotes the release of many chemical messengers such as dopamine. According to the results of this study, it seems that higher dose of NCT could be effective temporarily in increase of testicular sperm production.

Keywords: Methylphenidate, Nicotine, Rat, Sperm Count

P-20: Effect of Vitamin C and Vitamin E on Semen Parameters in Infertile Men

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Background: Infertility is an important medical and social

problem that has an impact on well-being. A significant development in the last 10 years in the study of human infertility has been the discovery that oxidative sperm DNA damage has a critical role in the etiology of poor semen quality and male infertility. It is now documented that vitamin C and vitamin E as a potent antioxidants protect the organism against oxidative stress via the inhibition of propagation of ROS reactions. In reproductive system, the antioxidant's role of this vitamin has also been reported to reduce testicular oxidative stress We explored the efficacy of vitamin c in combination with vitamin E for improving semen parameters in infertile men.

Materials and Methods: The study included 353 infertile men with idiopathic oligoasthenoteratospermia who received supplemental daily VitC (500 µg) in combination with Vit E (400 units) for at least 100 days. The mean age of cases was 28.5 years (range 20-45), and the median age was 30 years. These cases had presented with male factor infertility (primary or secondary) for at least 1 year. The longest and shortest duration of infertility was 10 years and 1 year, respectively. The median time of diagnosis of infertility was 1 year with a mean of 2.5 years.

Results: We observed 208 cases total improvement in sperm motility, morphology, count in comparison with no treatment. No response to treatment occurred in 145 cases after 14 weeks of combination therapy. On the basis of paired t-test results, combination therapy with oral Vit C and Vit E was effective for treatment of oligoasthenoteratospermia.

Conclusion: Supplemental VitC and Vit E may improve semen quality and have beneficial and protective effects, especially on sperm motility. We advocate their use for the treatment of idiopathic male infertility diagnosed with oligoasthenoteratospermia or asthenospermia in semen analysis.

Keywords: Sperm, Infertility, Vit C, Vit E, Oligoasthenoteratospermia

P-21: Assessment of Sperm Parameters and Protamine Deficiency in Fertile and Infertile Men Candidate for ICSI

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Background: Sperm nuclear and chromatin abnormalities are common amongst infertile men and are known to influence natural reproduction. Protamines cause sperm chromatin to compact. protamine deficiency in sperm is associated with DNA damage, low quality of sperm parameters and failed fertilization and pregnancy in infertile men candidate for assisted reproductive techniques, such as intra-cytoplasmic sperm insemination (ICSI). There for the aim of this study was to evaluate the semen parameters and global protamine deficiency between fertile and infertile men.

Materials and Methods: This is a cross-sectional study that cohort including total of 347 human semen samples were collected in the period from January 2016 to march 2018. men were divided to fertile (n=141) and infertile (n=206) (diagnosed with male factor infertility or participating in an assisted reproduction program) sperm parameters and protamine deficiency were assessed according to WHO (2010) protocol and Chromomycin A3 (CMA3) staining respectively. CMA3 staining is

one of the staining methods for detecting protamine deficiency in sperm nucleus.

Results: Sperm concentration, motility, volume, and normal morphology in infertile men were significantly decreased compared with fertile (P<0.05). The rate of CMA3-reacted spermatozoa (CMA3+) in infertile men was higher than fertile group (42.02 ± 18.29 vs. 25.00 ± 8.17, respectively, P<0.0001). The correlation between CMA3 positive spermatozoa and sperm parameters except sperm morphology shows significant association in infertile group (P<0.05), while in fertile group the correlation between CMA3 positive spermatozoa and sperm parameters except sperm morphology did not shows significant association (P>0.05).

Conclusion: It is concluded that the protamine content in sperm can be correlated to qualify of sperm parameters.

Keywords: Sperm Morphology, Sperm Concentration, Sperm Motility, Protamine Deficiency

P-22: Effect of Aloe Vera Gel on Mice Testes Treated with Doxorubicin

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Background: Chemotherapy drugs has temporary or permanent damage effects on testes in males. Doxorubicin is one of the most commonly anti-cancer drugs that used for cure of cancer. The effect of Doxorubicin is dependent on dose and has adverse effect on non-target tissue, such as the testes. Aloe vera is a perennial herbaceous cactus-like that may keep the testes healthy against the destructive effects of Doxorubicin and increase fertility. We studied the protective effect of Aloe vera gel on adverse effects of Doxorubicin on mice testes.

Materials and Methods: Eight week old male adult NMRI mice were divided into four groups. Control group; received nothing, Doxorubicin group: Doxorubicin was injected 4 times intraperitoneally weekly for 4 weeks (3 mg / kg body weight), Aloe vera group: received Aloe vera gel (200 mg / kg body weight) daily by intraperitoneal injection for 28 days and Aloe vera/Doxorubicin group: Doxorubicin and Aloe vera gel in the order mentioned above were injected intraperitoneally. After treatment the mice were sacrificed by cervical dislocation and testes and caudal epididym removed for histomorphometrical and sperm analysis.

Results: Our results showed that Doxorubicin reduced the number of primary spermatocytes, round and elongated spermatid and decreased tissue parameters such as testes diameter, seminiferous tubule diameter and epithelium thickness significantly compared to the control group, while Aloe vera increased all tissue parameters compared to the Doxorubicin group. Also Aloe vera increased significantly sperm count, viability and motility compared to the Doxorubicin group that decreased in Doxorubicin group.

Conclusion: We can say that Aloe vera has protective effect against the tissue damage caused by Doxorubicin on the mice testes.

Keywords: Doxorubicin, Aloe Vera, Testes, Mice

P-23: Relationship between Oligozoospermia and Expression of Sperm Phospholipase C ζ

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Background: Oligozoospermia refer to a semen with a low sperm concentration that commonly find in male infertility population. In recent years, several biomarkers are introduced as evaluation markers of sperm quality that diagnosis fertility potential in men. Phospholipase C zeta (PLC ζ) is considered as main sperm factor involved in oocyte activation that low or absence of this protein could be lead to low or failed fertilization in infertile men candidate for intracytoplasmic sperm injection (ICSI). Therefore, we aimed to evaluate and compare sperm parameters, PLC ζ at transcription level, and, DNA fragmentation between infertile men with normozoospermia and oligozoospermia.

Materials and Methods: Semen samples were collected from infertile men referring to Isfahan fertility and Infertility Center. After semen analysis according to world health organization (WHO-2010) criteria, semen samples with sperm concentration lower than 15 million per millilitre were considered for “oligozoospermia” group and semen samples with normal parameters in related to sperm concentration, motility, and morphology were considered for “normozoospermia” group. Then, expression of PLC ζ and DNA fragmentation were assessed using Real Time PCR [(data was expressed as threshold cycle (Ct)], and TUNEL assay, respectively. All of the statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS Inc., Version 23.0, Chicago, IL, USA) and Independent sample t-test was used for comparison of variations between two groups. P<0.05 was considered statistically significant.

Results: In this study, mean of sperm parameters such as sperm concentration, motility, and morphology were significantly lower in oligozoospermia than normozoospermia groups. In addition, mean percentage of DNA fragmentation was significantly lower in oligozoospermia than normozoospermia groups (P<0.05). Mean of PLC ζ CT was significantly higher in infertile men with oligozoospermia compared to normozoospermic men. Therefore, expression of PLC ζ was low in infertile men with oligozoospermia.

Conclusion: Low expression of PLC ζ in sperm from infertile men with oligozoospermia could be due to anomalies of gene expression that might be associated with pathogenesis in some subtypes of male infertility.

Keywords: Phospholipase C ζ , Oligozoospermia, DNA Fragmentation, Sperm Parameters

P-24: Antioxidant, Anti-Inflammatory and Testosterone Therapy Reinforces Spermatogonial Stem Cells Self-Renewal in Experimentally-Induced Varicocele; Possible Mechanisms

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Background: The varicocele (VCL) has been known as one of the infertility problems in 15-20% of the male population, which severely affects the spermatogenesis via inducing oxidative, inflammatory stresses and suppressing testicular endocrine potential. Thus, the antioxidant, anti-inflammatory and testos-

terone boosting chemicals (herbal and/or synthetic) have been considered as the alternative therapeutic methods. Thus, the VCL-induced damages can be divided into a-failed endocrine network between Leydig and Sertoli cells, b-the cytokines-induced effects on transcriptional factors and encoding genes, c-the oxidative stress-related molecular changes at cell cycle machinery.

Materials and Methods: To analyze mentioned three mechanisms, the experimental VCL was induced in Wistar rats, then the animals were divided into VCL-sole and antioxidant, anti-inflammatory and testosterone treated VCL-induced groups. Following 2 months, the animals were euthanized and the testicular glial cell line-derived neurotrophic (GDNF), its receptors Gfra1 and C-ret, the encoding active genes of spermatogonial stem cells (SSCs) self-renewal Bcl-6b and Etv5, and genes involving in cell cycle machinery including, Cyclin D1, CDK-4, p21, and the inflammatory mediators, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), cyclooxygenases (COX-II) and nitric oxide (NO), and the homeostatic factors heat shock protein70-2 (Hsp70-2), E2f1 expressions, serum levels of testosterone and inhibin B, the testicular total antioxidant capacity (TAC), malondialdehyde (MDA), glutathione peroxidase (GSH-px), superoxide dismutase (SOD), catalase, total thiol molecules (TTM) were investigated, using different RT-PCR, immunohistochemical, western blot and ELISA methods. the germinal cells DNA fragmentation was assessed using TUNEL staining. Moreover, the sperm parameters including, sperm count, viability, motility, DNA integrity, chromatin condensation were assessed. All results were compared between VCL-sole and treatment groups.

Results: Observations revealed that, administrating antioxidant and anti-inflammatory chemicals in association with testosterone boosting agents significantly ameliorates the VCL-impaired Leydig-Sertoli network, amplify the VCL-diminished GDNF, Gfra1, C-ret, Bcl-6b and Etv5 expression, and finally through this mechanism promote the SSCs self-renewal. Moreover, we showed that promoting the testicular endocrine and antioxidant system remarkably down regulates the DNA fragmentation, suppresses the p21 expression, amplifies the Cyclin D1 and CDK-4 expression, and through this mechanism promotes cell cycle progression in SSCs. More observations revealed a remarkable reduction in inflammatory mediators expression/synthesis/activity in treated groups. the animals in antioxidant and anti-inflammatory chemicals-treated groups exhibited enhanced testicular Hsp70, TAC, GSH-px, SOD, catalase and TTM levels and represented diminished E2f1 and apoptosis indices versus VCL-sole group. Finally, the VCL-treated groups exhibited improved sperm parameters compared to VCL-sole group.

Conclusion: The antioxidant and anti-inflammatory therapies in association with testosterone boosting agents (in sole and simultaneous form of administration) promote the Leydig-Sertoli cells physiologic interactions, which in turn a- amplifies the Sertoli-related niche factors expression/synthesis and affect on SSCs self-renewal, b- downregulates the inflammatory mediators expression/synthesis and affect on SSCs self-renewal, c- reduces DNA fragmentation both at germ cells and sperm levels and amplifies the homeostatic factors Hsp70-2 expression and suppresses the E2f1 protein level and d- improves the sperm parameters resulting in enhanced fertilization potential.

Keywords: Varicocele, Spermatogonial Stem Cells, Self-Renewal, Cell Cycle

P-25: Evaluation of Seminal Plasma Zinc Level in Astheno-

zoospermic and Normozoospermic Men

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Introduction: Zinc is known as a crucial trace element for wide range of biological activities. It plays a pivotal role in normal function of reproductive system such as testis development and sexual maturity. In addition, importance of Zinc in sperm concentration, morphology and DNA integrity is well known. In limited number of studies association of seminal plasma Zinc level and sperm motility have been evaluated, although precise role of seminal plasma Zinc in sperm motility have not been fully understood. Therefore, the aim of this study is comparison of seminal plasma Zinc levels between normal and low motility semen samples to elucidate the role of Zinc in sperm motility.

Methods: Semen sample were collected during 2017-2018 by masturbation from 211 asthenozoospermic (total motility < 40%) and 209 normozoospermic (total motility > 40%) men (according to WHO 2010 criteria) who are referred to the Royan Institute for ART cycles. Subsequently, sperm motility were analyzed by computer assisted sperm analyzer (CASA) system. After that, semen samples were centrifuged and seminal plasma were transferred to conical microtube. Finally, the Zinc level of seminal plasma was examined by spectrophotometry. Data were analyzed by independent T-test of SPSS software and Pearson correlation coefficient were used to evaluation of sperm motility and seminal Zinc level.

Results: The seminal plasma Zinc level was not significantly different in the normozoospermic and asthenozoospermic groups. Moreover, increasing the seminal plasma zinc level caused the reduction in total and progressive sperm motility in normozoospermic men. ($P < 0.05$). However only the progressive motility exhibits negative correlation with Zinc level in asthenozoospermic patients ($P < 0.05$).

Conclusion: Our result indicated that no difference was observed in seminal plasma Zinc level among normozoospermic and asthenozoospermic individuals. Furthermore, seminal plasma Zinc level showed negative correlation with progressive motility in both normal and low motility semen samples.

Keywords: Zinc, Asthenozoospermia, Sperm motility, Seminal plasma

P-26: Intratesticular Injection of Zinc Oxide Nanoparticles Versus Surgical Castration to Sterilize Male Rats

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Background: Male sterilization by chemical agents is a non-surgical contraceptive approach designed to induce azoospermia and, therefore, infertility by light and transmission electron microscopy, the efficacy of a single intratesticular injection of a chemical agent a sterilize for male rats. The objective of the present study was to evaluate, sperm count, sperm motility, sperm morphology, DNA integrity and Teratozoospermia Index were evaluated after intratesticular injection of Zinc Oxide Nanoparticles in rats.

Materials and Methods: Sixty adult male Wistar rats were divided into 6 groups randomly. After 10 days of acclimation time, the animals were sedated by xylazine, ketamine cocktail. The testicle diameter related volume of Zinc Oxide Nanoparticles (with different concentrations of 5, 10 and 25 mg/ml), zinc gluconate (25 mg/ml) and sterile distilled water were injected via the intratesticular method in each group separately. Rats on experimental and control groups were killed under anesthetic conditions after 30 and 60 days. sperm count, sperm motility, sperm morphology, DNA integrity and Teratozoospermia Index were measured.

Results: The sperm count, sperm motility, sperm morphology in Zinc Oxide Nanoparticles group were significantly lower than the control group ($P < 0.05$). On the other hand, DNA integrity in Zinc Oxide Nanoparticles group was significantly higher than zinc oxide and distilled water groups ($P < 0.05$). Also, Teratozoospermia Index was significantly increased in Zinc Oxide Nanoparticles group ($P < 0.05$).

Conclusion: Low cost, ease of use, and cultural acceptance of a castration technique that does not require removal of the testes make Zinc Oxide Nanoparticles a valuable option for large-scale use in animals, particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons and staff. Notably, Intratesticular injection of Zinc Oxide Nanoparticles solution impaired spermatogenesis in rats and has great potential as a permanent sterilize in this species.

Keywords: Zinc Oxide Nanoparticles, Sterilize, Intratesticular, Rats

P-27: Histomorphometric and Biochemical Analysis of Protective Effects of Rosa Canina L. Against Alcohol Induced Testicular Damages

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Background: Ethanol consumption induces histological and physiological changes in reproductive system of mammals. Rosa canina L. (RC) have antioxidative and anti-inflammatory effects in various oxidative stress conditions. In this study, it was aimed to examine the effects of RC effects on ethanol induced testicular damages.

Materials and Methods: In our study, 24 male Wistar rats were divided into four groups: control, ethanol, ethanol + RC (500 mg kg⁻¹) group and RC (500 mg kg⁻¹). After the treatment, mean seminiferous tubule diameter (MSTD), Johnson's mean testicular biopsy score (MTBS) criteria were used for histological evaluation. In addition sperm parameters including: sperm count, motility, viability and morphology were analyzed. For biochemical analysis, total protein level, Superoxide

dismutase (SOD) activity, Malondialdehyde (MDA) and H₂O₂ levels were evaluated.

Results: Our data indicate a significant reduction in the sperm count, motility, viability in ethanol group but RC ameliorate this reduction in RC + ethanol group. In this study both MSTD and MTBS values were decreased in ethanol treated rats. However, RC treatment was associated with increase in both of these values. SOD activities significantly increased in the animals ingested with RC extract prior to ethanol compared to the ethanol group. Decrease of MDA and H₂O₂ levels were statistically significant in the animals that ingested the RC extract prior to ethanol compared to the ethanol group.

Conclusion: Our study showed that the reproductive toxicity caused by ethanol may be prevented by RC treatment.

Keywords: Ethanol, Testis, Rosa Canina, Rat

P-28: The Study of Microscopic Structure of Testicular Seminiferous Tubules Following The Administration of Monosodium Glutamate in Paclitaxel Treated Adult Mice

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Background: Chemotherapy is one of the risk factors which affect the fertility. Paclitaxel (PTX) is an effective chemotherapeutic agent and mitotic inhibitor which binds to DNA and prevents the DNA replication. PTX is used against a wide range of solid tumors. It has been reported numerous functional and structural alterations in testicular tissue following the administration of PTX. Monosodium glutamate (MSG) is the L-form of glutamic acid. Some studies indicated that monosodium glutamate can induce oxidative stress and has toxic effects on human and animal's tissues. Male infertility and alteration of sperm production and morphology are the most reported changes in cases of MSG administration. In this study the effects of coadministration of PTX and MSG on the microscopic structure of seminiferous tubules (STs) was evaluated.

Materials and Methods: Paclitaxel (2 mg/kg i.p.) was administered to mice once per day for 5 consecutive days. MSG (30 & 60 mg/kg i.p.) was administered for 28 days before or after PTX administration. Formaldehyde fixed testicular tissue samples were stained with hematoxylin and eosin method for quantitative evaluation of STs.

Results: The mean diameter of STs was reduced significantly in all groups in comparison to control group. Coadministration of PTX and MSG dose dependently led to more reduction in STs diameter. Tubular atrophy was observed in more degree following the administration of MSG after PTX.

Conclusion: Structural changes in testicular STs, following the administration of PTX can be related to fertility alterations. Oxidative compounds such as MSG can induce more structural and functional alterations of testicular tissue in cases of chemotherapies.

Keywords: Mice, Monosodium Glutamate, Paclitaxel, Seminiferous Tubules

Animal Biotechnology

P-29: The Effect of Omega 3-6-9 on CASA Parameters of Buffalo Epididymal Sperm in HTF Cell Culture Medium

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Background: Mammalian sperm contains very high proportion of PUFA (polyunsaturated fatty acids), especially Docosahexaenoic acid (DHA) and docosapentaenoic acid. DHA is an important fatty acid for sperm motility and viability. There are positive correlations between DHA and the proportion of motile spermatozoa in humans, boars, goats, rams and bulls. It has been known sperm cells can be damaged by the process of cooling and freezing in cryopreservation and this is related to the disruption of membrane lipids resulting in mitochondrial damage and loss of integrity of acrosomal and plasma membranes. These events cause a loss and decline in motility and viability. Different ratios of n-3/n-6 PUFAs improved sperm quality and increasing n-3/n-6 PUFA ratio, sperm density and motility were increased. In fact, very few studies have been conducted to examine the effects of the ratio of n-3/n-6 PUFAs on male sperm cells.

Materials and Methods: In this research, sperms were collected from 20 pairs of male buffalo testicles. Collection was carried out in slaughtered animals. Sample processing was performed in a walk-in fridge (5°C) immediately after its arrival to laboratory. Then tail of epididymis was fixed between two fingers. Sperm cells were collected performing several incisions in the caudal epididymis with a surgical blade. Four levels of omega-3, 6, 9 (0.25-0.5-1-3- mM) were added into human tubal fluid containing sperms (40×10⁶ sperm/ml), with 10% bovine serum albumin and were kept for 36 hours at 5°C. Sperm motility was examined at 1, 12, 24, 36, hours after kept in refrigerator at 5°C with computer assisted semen analysis (CASA).

Results: Data were analyzed using SPSS 25 statistical package and presented as mean ± SEM. Multiple comparisons by post-hoc test such as tukey and tamhane were performed to determine statistical differences among groups. Results were considered significant at P<0.05 for all tests. Analysis of data revealed that major CASA parameters such as rapid progressive motility (Class A %), progressive motility (Class B %), progressive motility (Class A+B+C %), velocity curvilinear (VCL μm/s), velocity average path (VAP μm/s), straight line velocity (VSL μm/s) and linearity (LIN %) were not significantly changes in omega-3,6,9 fatty acids treated groups as compared to control.

Conclusion: These findings demonstrated that omega -3, 6, 9 at these levels had no desirable effect on motility patterns of buffalo epididymal sperm measured by CASA during storage at 5°C.

Keywords: CASA, Buffalo, Omega-3,6,9, Epididymal Sperm

P-30: Superovulated Environment Can Negatively Change The Implantation Rate, Fetal and Placental Weight and Histomorphological Parameters of Placenta

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Background: Several studies have demonstrated that assisted

reproductive techniques (ARTs) have been associated with several perinatal and postnatal defects. Superovulation is an integral aspect of ARTs which induces significant changes in hormonal environment of uterus.

Materials and Methods: This study used mouse as a model, aimed to investigate the effects of superovulation on resorption rate, fetal and placental weight and histomorphological parameters of placenta at embryonic day 15.5 (E15.5).

Results: Our results revealed that resorption rate in superovulation group was significantly higher than control group (35.1% vs. 2.775%, respectively) ($P < 0.05$). In addition, we found that superovulation resulted in significantly reduced placental weight (PW) (65.76 ± 4.04), fetal weight (FW) (194.24 ± 20.16) and PW/FW (0.34 ± 0.04) in compare to control group (90.31 ± 3.06 , 365.08 ± 12.06 and 0.24 ± 0.02 , respectively) ($P < 0.05$) at E15.5. Finally, the superovulation placentae exhibited significantly smaller junctional zone and bigger labyrinth zone against to control group ($P < 0.05$).

Conclusion: Our results revealed that superovulation creates a hormonal environment that negatively affected resorption rate, fetal and placental weight. Furthermore, superovulation changed the histomorphometrical characteristics of placental tissue. Together, these results may have implications for treating infertility in humans.

Keywords: ART, Superovulated, Mouse, Placental

P-31: The Effects of Heat Stress during *In Vitro* Maturation Process on The Developmental Competence of Ovine Oocytes

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Background: Evidences show that farm animals such as cattle, pigs, and sheep have lower fertility in the summer than in any other season. Recent studies demonstrate that heat stress causes infertility not only by affecting hormonal secretion and embryo development but also by damaging the oocyte. The aim of this study was evaluating impacts of heat stress during the *in vitro* maturation (IVM) process on the developmental competence of ovine oocyte.

Materials and Methods: Good quality cumulus-oocyte complexes recovered from the aspirates of the follicles of ovine ovaries were cultured in the droplets of *in vitro* maturation medium. The oocytes were subjected to the heat stress of 41°C during the first 6 (F6-41) and 12 (12F-41) or the last 6 (L6-41) and 12 (L12-41) hours of IVM period. After fertilization of oocytes and cultur of resulted embryos, cleavage and blastocyst formation were observed and recorded. The data were analyzed using one-way ANOVA followed by turkey's post-hoc test. The data were expressed as mean \pm SEM and the differences at the level of $P < 0.05$ were considered significant.

Results: Results showed that duration of hyperthermic treatment was the determinant factor of oocytes' response to the heat stress treatment. There was no significant difference between the cleavage rates of hyperthermic treatment groups and the control group although heat stressed groups had lower cleavage rate than the control group. Although the blastocyst rates of 6F-41 and 6L-41 groups were lower than the control group, these differences were not significant. The blastocyst rates of 12F-41 and 12L-41 groups ($23.8 \pm 4.93\%$ and $31.8 \pm 4.33\%$,

respectively) were significantly lower than that of the control group ($54.6 \pm 4.42\%$).

Conclusion: This study showed that heat stress during the IVM period of ovine oocytes decreased the developmental competence of oocytes and this decrease increased by increasing the time of hyperthermic exposure.

Keywords: Oocyte, Heat Stress, Developmental Competence

P-32: The Impacts of Heat Stress during *In Vitro* Maturation Process on The Nuclear Maturation and Occurrence of Apoptosis in Ovine Oocytes

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Background: The aim of this study was evaluating impacts of heat stress during the *in vitro* maturation (IVM) process on the nuclear maturation and occurrence of apoptosis in ovine oocytes.

Materials and Methods: Good quality cumulus-oocyte complexes recovered from the aspirates of the follicles of ovine ovaries were cultured in the droplets of *in vitro* maturation medium. The oocytes were subjected to the heat stress of 41°C during the first 6 (F6-41) and 12 (12F-41) or the last 6 (L6-41) and 12 (L12-41) hours of IVM period. After 24 hours, assessment of apoptosis and nuclear maturation was performed simultaneously on the same oocytes. For detection of apoptosis the Annexin-V-FLOUS Staining Kit (Roche Diagnostics, Mannheim, Germany) was employed according to the manufacturer's instructions, and for chromatin staining 5 μ g/ml of Hoechst 33342 was added to the binding buffer. The data were analyzed using one-way ANOVA followed by turkey's post-hoc test. The data were expressed as mean \pm SEM and the differences at the level of $P < 0.05$ were considered significant.

Results: Results showed that necrotic oocytes did not statistically differ between the heat stressed groups and control group. Apoptosis rates in 12F-41 and L12-41 groups were significantly different in comparison to the control group (16.0 ± 3.85 and 14.4 ± 3.78 vs. $3.6 \pm 0.70\%$). The oocytes with fragmented chromatin in 12F-41 and 12L-41 groups (11.8 ± 2.31 and $14.3 \pm 3.10\%$ vs. $1.6 \pm 0.69\%$), and maturing oocytes in 12F-41 (65.8 ± 4.83 vs. $86.8 \pm 2.36\%$) were significantly different in comparison to the control group.

Conclusion: This study showed that nuclear maturation and apoptotic status of ovine oocytes were affected by relatively long periods of hyperthermic treatment.

Keywords: Oocyte, Heat Stress, Nuclear Maturation, Apoptosis

P-33: The Effect of Hydroalcoholic Extract of Nettle Leaves on The Occurrence of Miosis in NMRI Mice

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Background: The Oocyte is a unique cell in woman's body, not only in its special structure and function, but it is the only cell that undergoes meiosis. *in vitro* maturation (IVM) is an attrac-

tive treatment alternative for women with various forms of infertility without recourse to ovarian stimulation. Approximately 2,000 healthy infants have been born following immature oocyte retrieval and IVF. Therefore, this study has been conducted with the aim of the efficacy of hydroalcoholic hackberry leave extract on *in vitro* maturation of premature mice ovum.

Materials and Methods: In this study, female mice aged 6-8 week, were used. 48 hours after injecting PMSG hormone, when ovaries aren't still mature, mice were killed by cervical dislocation and ovaries were removed. The hackberry extract was added to the experimental groups by the concentrations of 250, 500, 1000, 2000 mg/ml and was incubated in CO₂ incubator for 24h. The stages of ovum maturity were examined and SPSS software was used to analyze of data.

Results: The average percentage of MI oocytes in the experimental group with a concentration of 2000 mg/ml showed a significant increase and decreased in other experimental groups. The average percentage of MII oocytes in the experimental group with the highest concentration of 500 micrograms per mg showed a significant increase compared to the control group. Its rate increased in all experimental groups, but decreased significantly between the control and experimental groups at a concentration of 2000 mg/ml.

Conclusion: In general, the results of this study showed that there were more maturational concentrations and the oocyte maturation rate should be reduced at high concentrations.

Keywords: Oocyte, *In Vitro* Maturation, Hydroalcoholic Hackberry, MI Oocytes, MII Oocytes

P-34: Effect of Rosiglitazone on *In Vitro* Pre-Implantation Mouse Embryo Development

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Background: Rosiglitazone as an anti-diabetic drug has anti-inflammatory and anticancer properties. Rosiglitazone are highly selective and potent agonists for the nuclear receptor Peroxisome proliferator-activated receptor gamma (PPAR γ), strongly implicated in female reproduction. Rosiglitazone have been proposed to exert anti-inflammatory effects because they may be inhibit secretion pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin 6. This study aimed to investigate the effect of rosiglitazone on *in vitro* pre-implantation mouse embryo development.

Materials and Methods: 6-8 weeks female NMRI mice were superovulated with PMSG by intraperitoneal injection, followed by HCG injection and mating after 48 hours. Mice were killed via cervical dislocation 18 hours post HCG injection. Embryo were then recovered from oviduct ampulla and cultured with and without rosiglitazone (0, 10, 25, 50, 75 μ M). Cleavage and blastocyst rate were assessed. Also, total cell number were assessed with differential staining.

Results: 10 μ M rosiglitazone caused the highest cleavage (% 100) and blastocyst rates (%58.9795) in comparison to other concentrations (25, 50, 75 μ M) but not significant with control. Also, there is no significant difference in the total cell number

between control and rosiglitazone groups. Blastocyst quantity and quality reduced in 50, 75 μ M groups in comparison to control.

Conclusion: These data provide evidence that PPAR γ can be a key target for embryo development and high concentration of PPAR γ agonist (rosiglitazone) is toxic.

Keywords: Mouse, Rosiglitazone, Blastocyst, PPAR γ

P-35: Effects of Cerium Dioxide Nanoparticles on *In Vitro* Fertilization and Embryo Development in Mice

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Background: Cerium dioxide nanoparticles (CeO₂NPs) are widely used in industry and medicine and are also used in as additives diesel to reduce fuel consumption and CO₂ gas emissions. According to organization for economic cooperation and development, CeO₂NPs are in the priority list nanoparticles required argent evaluation. In the current *in vitro* study, the effects of CeO₂NPs on *in vitro* fertilization and embryos developing rates were detected.

Materials and Methods: CeO₂NPs at two different concentrations, (10,100) were added to fertilization culture medium and then *in vitro* fertility, embryo development (two-cell embryos were evaluated following 12h.

Results: The results showed that *in vitro* CeO₂NPs exposure caused significant reduction in the percentages of fertilization and embryo development (two-cell embryo blastocyte formation) and also increase the rate of arrested embryos in comparison to control.

Conclusion: Decreased fertilization rates may result from: 1. CeO₂NP's genotoxicity on gametes, 2. a mechanical effect, disrupting gamete interaction, and 3. oxidative stress induced by CeO₂NP. The data obtained from its study indicated that CeO₂NPs are toxic to the fertility and embryos development and precaution should be taken about human exposure.

Keywords: Nanoparticles, Ceriumdioxide, *In Vitro* Fertilization, Embryo Development, Mice

P-36: Attenuate The Toxic Effect of Ethanol during Oocyte Maturation with Alpha-Lipoic Acid in Sheep

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Background: Alcohol abuse during unprotected sexual intercourse may have devastating effect on health of future progeny through affecting the quality of oocyte and embryo for further development. Previous studies have shown that alpha-lipoic acid (ALA) as an antioxidant has protective effect against al-

cohol toxicity.

Materials and Methods: In this study ovine cumulus oocyte complexes (COCs) were matured in control, ALA (25 μ M) + ethanol (1% (v/v)) and ethanol groups. Cumulus expansion index, ROS and GSH level were assessed after oocyte maturation. Subsequently, developmental competence of matured oocytes was assessed in terms of cleavage and blastocyst rates. Finally, DNA fragmentation and gene expression were analyzed in resultant blastocysts.

Results: The results revealed that alcohol significantly reduced cumulus expansion index, blastocyst yield and rate of apoptosis in resultant blastocysts. Addition of 25 μ M ALA to 1% alcohol during oocyte maturation decreased ROS level and elevated GSH content. Furthermore, supplementation of maturation medium with ALA attenuated the effect of 1% ethanol and significantly increased the blastocyst formation and hatching rate in compared to control and ethanol groups. In addition, quality of derived blastocysts in ALA + ethanol was improved based on TUNEL positive cells, mRNA expression of apoptotic, pluripotent and anti-oxidant markers.

Conclusion: Our results suggest that ALA not only overcomes the negative effect of alcohol toxicity during oocyte maturation but also improved blastocyst yield and quality of resultant embryos. Therefore, ALA as a food supplement, or as a chemical highly available in green vegetable, is recommended for couples planning to achieve pregnancy and is highly recommended for individuals at risk of alcohol abuse with possible unprotected sexual intercourse who may not have also healthy eating habits.

Keywords: Alpha Lipoic Acid, Alcohol, Oocyte Maturation, ROS, Apoptosis

P-37: Effects of Chick Embryo Extract on Cx 43, Cx 37, Bmp6 and, Gdf9 Genes Expression *In Vitro* in Preantral Follicles Isolated from Vitrified and Non-Vitrified Mouse Whole Ovary

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Background: Currently, vitrification of the ovarian tissue is used as an effective way to preserve oocytes and follicles. It appears to be the case that chick embryo extract (CEE) can enable satisfactory results in puberty of prenatal follicles.

Materials and Methods: For this study, ovaries of 10-12 day old Naval Medical Research Institute (NMRI) female mice divided into two groups: non-vitrified and vitrified. The pre-antral follicles with a mean average diameter of 110-130 μ m were isolated by mechanical methods of non-vitrified and vitrified ovaries and cultured in the aforementioned groups. First to second groups consisted of: non-vitrified ovaries in base solution (first group), base solution with 5% chick embryo extract

(second group), and third to fourth groups consisted of vitrified-warmed ovaries in base solution (third group) base solution plus 5% chick embryo extract (fourth group). The evaluations included the survival rate of the follicles, the antrum formation rate, and maturation rate of oocyte that reached the metaphase two stage. Estradiol hormone levels, survival of follicles, morphology of oocytes, and ovarian tissue histology were evaluated. Also, quantitative expression of oocyte maturation genes (Cx43, Cx37, Bmp6, and Gdf9) was evaluated after 24 hours and 12 days of culture.

Results: The amount of antrum formation was significantly higher in the 5% chick embryo extract in vitrified and non-vitrified groups than other groups (P<0.05). The Connexin 43 gene *in vitro* cultured group, with 5% chick embryo extract, showed an analytically higher expression than the control group (vitrified, P<0.05).

Conclusion: Considering the significant difference in the antrum formation and gene expression of Connexin 43 between the CEE and control groups, it seems that CEE could improve the quality and quantity of matured follicles *in vitro* but the, other ovarian functional factors assays, including fertilization ability and developmental potential of derived embryos are necessary.

Keywords: Preantral Follicle, Chick Embryo Extract, Ovarian Tissue, Vitrification, *In Vitro*

P-38: The Effect of OCT4 Activating Compound-1(OAC1) on Nuclear Reprogramming of Bovine Somatic Cell Nuclear Transfer (SCNT) Embryos

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Background: Although somatic cell nuclear transfer (SCNT) is a promising technology, that its potential usage is hampered by various developmental failure during pre- and post-implantation development. The abnormal expression of oct4 in SCNT embryos beside to its central role in the reprogramming process prompted us to ask whether Oct4-activating compounds (OAC1) may enhance reprogramming efficiency in SCNT embryos.

Materials and Methods: Bovine fibroblast cells were treated with 1.5 and 3 μ M OAC1 compound for 6 days in presence of 10% FBS. Cell proliferation and expression of OCT4 were assessed in treated cells along with their control. Furthermore, treated cells were used for SCNT procedure as donor cells to assess the effect of OAC1 treatment in developmental competence of SCNT embryos in terms of cleavage and blastocyst rates.

Results: The treatment of these cells with 1.5 and 3 μ M OAC1 did not affect expression of OCT4 after 6 days. Furthermore, our results revealed that treatment of donor cells with OAC1 did not enhance cleavage and blastocyst rate of reconstructed oocytes.

Conclusion: Our results revealed that treating of bovine fibroblast cells with OAC1 neither increase OCT4 expression of fibroblast cells nor developmental competence of bovine SCNT embryos. These data are in contrast with the data which demonstrated that OAC1 enhance iPSCs formation in mouse, which may be due to species difference, which needs more investigat-

tion to be elucidated.

Keywords: Reprogramming, SCNT, OCT4, OAC1

P-39: Meiotic Progression of Sheep Oocytes from Preantral Follicles with Two Different Sizes

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Background: Oocyte nuclear and cytoplasmic maturation are gradually acquired during folliculogenesis *in vivo*. One of the oocyte *in vitro* maturation challenges is heterozygous population of oocytes which have different nuclear and cytoplasmic potential. We aim to investigate meiotic progression of sheep oocytes from preantral follicles with two different sizes.

Materials and Methods: Cumulus oocyte complexes were isolated by puncturing 2 to 4 mm and 4 to 6 mm diameter follicles, and then were cultured for 0 to 24h maturation in M199. For assessing meiotic progression, oocytes were fixed in 4% paraformaldehyde and stained with Hoechst. Meiotic progression was classified into germinal vesicle (GV), germinal vesicle breakdown and metaphase I, II stages. The percentages of GV in different hours of maturation were compared between two follicular sizes.

Results: In 2-4 mm group, the percentage of GV oocyte in 2, 4, 6, 8, 10, 12, and 24 h of maturation was 92.74, 94.4, 98.33, 76.31, 53.40, 33.8, 24.30, and 20.95, respectively. The corresponding percentage for 4-6 mm group was 97.33, 96.67, 96.67, 78, 67.67, 54.33, 34.33, and 20.33, respectively. Rescue of meiosis occurs faster in oocytes with 2-4 mm follicular diameter as it is observed that after 10h of maturation 53.40% of 2-4 mm group and 67.67% of 4-6 mm group remain in GV state ($P < 0.05$).

Conclusion: This study provides evidence that different follicular stages have different meiotic progression, thus the selection of appropriate follicles for *in vitro* maturation requires further study.

Keywords: Meiotic Progression, Preantral Follicles, Maturation, Oocyte

P-40: Fetal Bovine Serum and Bovine Serum Albumin on Sperm Capacitation

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Background: To keep spermatozoa mobility for a long time and to evaluate the migration and fertilization, it is necessary to use a basic medium with nutrient factors. For this purpose, fetal bovine serum (FBS) is often used to maintain cell growth. The aim of this study is to find out more about the effect of allogeneic mouse serum (AMS) as compared with FBS and BSA on sperm capacitation.

Materials and Methods: Caudal epididymis of mice was extracted and transferred to MHRM medium with 0, 5, 10, 15, 20 percentages of AMS, FBS and BSA. After treatment, MTT assay was performed to evaluate sperm viability. Also, the rate of progression and sperm motility was counted and analyzed by light microscope.

Results: The MTT assay showed that sperm had the highest viability in 15% BSA. Also, the rate of progression in the medium containing 10% BSA and 10% FBS showed the highest percentage of progression and the highest percentage of motility was observed in medium containing 10% FBS and 5% AMS. In addition, there was a significant difference ($P < 0.05$) between these groups and the control group.

Conclusion: Different types of serum are added to the medium as a supplement to enhance the quality of culture. The results indicate that albumin in the serum reduces cholesterol in the sperm membrane. The presence of serum and serum type are generally important for sperm capacitation *in vitro*. Moreover, low serum percentages show better results rather than other percentages in sperm capacitation.

Keywords: Capacitation, Allogeneic Mouse Serum, Fetal Bovine Serum, Bovine Serum Albumin, MTT

P-41: Effects of Chick Embryo Extract on Gdf9, Bmp6, Cx37, Cx43 Genes Expression in Non-Vitrified and Vitrified Mouse Preantral Follicles during *In Vitro* Culture

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Background: Vitrification and *in vitro* culture of preantral follicles are a suitable way to female's fertility preservation that are exposed to the infertility risks. The development of preantral follicles culture system can increase the growth rate and quality of oocytes. To this aim, the effects of various biofactors such as proteins, sugars and other factors have been investigated. Chick embryo extract (CEE) contains various factors such as growth factors, vitamins, mineral, sugar, protein and lipids, can stimulate growth and differentiation of the cells. In this research, the effects of chick embryo extract on mouse preantral follicles *in vitro* culture has been investigated.

Materials and Methods: For this study, preantral follicles with a diameter of 110-130 μm were isolated from 12-14 days old female mouse and divided and *in vitro* cultured 12 days into two groups of Fresh (non-vitrified) and Vitrified. The base culture medium (BM) contains: α -MEM + 5% FBS + 1% ITS (5mg/ml) + 1% FSH. Each vitrified and non-vitrified follicles were classified into 4 groups: 1. Fresh BM group: culture of non-vitrified preantral follicles in the base medium 2. Fresh CEE group: culture of non-vitrified preantral follicles were in a base medium of + 5% chick embryo extract 3. Vit BM group: culture

of vitrified-warmed preantral follicles in the base medium and 4. Vit CEE group: culture of vitrified-warmed preantral follicles, in base medium + 5% chick embryo extract. The follicles were cultured for 12 days after melting in sucrose solutions with decreasing concentrations. Survival rates were measured on days 1, 4, 8 and 12 after culture, as well as expression of Gdf9, Bmp6, Cx43 and Cx37 genes on the 12th day after culture in all groups.

Results: The results of this study showed the lowest survival of follicles during different days of culture in Vit BM group, compared to Fresh CEE group ($P<0.05$). Antrum formation in Vit CEE and Fresh CEE groups was significantly higher than Vit BM and Fresh BM groups respectively ($P<0.05$). The expression of Cx43 gene on the 12th day in the Vit CEE group was significantly higher than Vit BM group ($P<0.05$).

Conclusion: Therefore, chick embryo extract as a natural nutritional supplement can be used in vitrification and *in vitro* culture of mouse preantral follicles in future study.

Keywords: Chick Embryo Extract, Preantral Follicle, *In Vitro* Culture, Vitrification

Changes in Serum Testosterone and Alkaline Phosphatase Activity in Testis Tissue after Administration of Berberine in Experimental Varicocele

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Background: Varicocele (VCL) is one of the greatest andrological pathologies since 35% to 40% of male subfertility is due to varicocele. Serum levels of testosterone in varicocele significantly reduced. In addition, alkaline phosphatase activity in testis tissue in varicocele increases. Present study was done in order to evaluate the changes in serum level of testosterone and evaluate alkaline phosphatase activity in testis tissue after administration of berberine in experimental varicocele.

Materials and Method: Thirty mature male Wistar rats were randomly divided into control (NO: 6 rats), control-sham (NO: 6 rats) and experimental groups (NO: 18 rats). The animals in experimental groups were undergone experimental varicocele and simple laparotomy was conducted in control-sham group. The experimental group subdivided into: Non-treated VCL-induced, 50 mg/kg and 100 mg/kg berberine-treated groups. The serum levels of testosterone and alkaline phosphatase activity in testis tissue were assessed.

Results: Observations revealed a significant ($P<0.05$) reduction in serum levels of testosterone and an significant ($P<0.05$) increase in alkaline phosphatase activity in testis tissue in non-treated VCL-induced animals versus control and control-sham groups. No significant changes were found between control and control-sham groups. Meanwhile, the berberine-treated animals (especially 100 mg/kg) exhibited a remarkable ($P<0.05$) enhancement in Serum levels of testosterone and a significant ($P<0.05$) reduction in alkaline phosphatase activity in testis tissue.

Conclusion: According to our finding, Berberin by increasing in serum levels of testosterone increases the Testicular endocrine capacity and protects Leydig cell against Inflammatory

and oxidant injury varicocele. In addition, Berberin by decreasing in alkaline phosphatase activity in testis tissue, reduces inflammatory damage of varicocele.

Keywords: Varicocele, Berberine, Testosterone, Alkaline Phosphatase

Effect of Berberine administration on male rat reproductive function: Evaluation of serum FSH and LH in experimental varicocele 3

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Background: Varicocele (VCL) is characterized with abnormal tortuosity of the veins of the pampiniform plexus, which drains the testicular tissue. This impairment is one of the physical causes of infertility in men. Gonadotropin releasing hormone (Gnrh) secreted by the hypothalamus elicits the release of gonadotrophins i.e follicle stimulating hormone (FSH) and lutenizing hormone (LH) from the pituitary gland. FSH binds with receptors in the sertoli cells and stimulates spermatogenesis. LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis. serum levels of FSH and LH in varicocele significantly increase. Present study was done in order to evaluate the changes in serum levels of FSH and LH after administration of berberine in experimental varicocele.

Methods and Materials: Thirty mature male Wistar rats were randomly divided into control (NO: 6 rats), control-sham (NO: 6 rats) and experimental groups (NO: 18 rats). The animals in experimental groups were undergone experimental varicocele and simple laparotomy was conducted in controlsham group. The experimental group subdivided into: Non-treated VCL-induced, 50 mg/kg and 100 mg/kg berberine-treated groups. The serum levels of FSH and LH were assessed.

Results: Observations showed a significant ($P<0.05$) enhancement in serum levels of FSH and LH in non-treated VCL-induced animals against control and control-sham groups. No significant changes were found between control and control-sham groups. However, the berberine-treated animals (especially 100 mg/kg) represent a remarkable ($P<0.05$) enhancement in Serum levels of FSH. There was no significant change in Serum levels of LH between Non-treated VCL-induced and 50 mg/kg and 100 mg/kg berberine-treated groups.

Conclusion: Considering these findings, berberine decrease serum levels of FSH. Probably increase in serum levels of testosterone resulting from administration of berberine, as previously reported, reduced serum levels of FSH. In addition, berberine reduced serum level of LH, but this reduction was not significant.

Keywords: Varicocele, Berberine, FSH, LH

Embryology

P-42: Heat Killed *Tsukamurella Incheonensis* Improve Sperm Parameters in Mice by Reduction of Oxidative

Stress in Epididymis

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Background: The issue of male fertility and nutrition is a complicated matter but it has been shown that some nutrients and supplements can improve male fertility. Some of aerobic Actinomycetales species, including *Tsukamurella inchonensis* (TI) are capable of having beneficial effects on animal and human health when used as suspensions of killed bacilli. The nutritional benefits of probiotics have been well documented, but studies on the effect of them on sperm parameters and male fertility are lacking. This study was conducted to evaluate the effects of heat killed preparation of TI on sperm quality and quantity parameters and its relation with the state of oxidative stress of epididymis.

Materials and Methods: In this study, 20 adult male mice were divided into 4 groups include control, treatment1, 2 and 3. Control group received normal saline and treatment groups received 5×10^7 , 10^8 and 2×10^8 CFU/day of *T. inchonensis* in normal saline by oral gavage for eight consecutive days, respectively. Finally animals were euthanized, and the epididymis was removed and dissected in Ham's F10 solution and incubated at 37°C for sperm analysis. Total sperm count, sperm motility and viability were assessed according to the WHO standard methods. Epididymal tissue samples were homogenized with cold ice 1.15% KCl to creat 10% homogenate and stored at -80°C until its glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured. Finally, data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc ($\alpha=0.05$).

Results: Sperm analysis indicated that TI had no side effect on spermatogenesis and increased quality and quantity of sperm in dose dependent manner. Results showed that changes in low dose of TI were not significant compared to control group but percentage of rapid progressive motile sperms was increased significantly in mid ($P<0.05$) and high ($P<0.01$) doses of TI compared to control groups. Our results indicated that high dose of TI increased sperm viability significantly compared to control group ($P<0.001$). Analysis of oxidative stress indices revealed that TI reduced level of MDA and increased GPx, SOD and TAC level of epididymal tissue in a dose dependent manner but this changes was significantly only in high dose of TI compared to control group ($P<0.05$).

Conclusion: Based on our results it can be concluded that administration of TI as a probiotic supplement or additive can be effective and suitable way to increase male fertility by decreasing of oxidative stress in epididymal tissue and improve quality and quantity parametes of sperm.

Keywords: Epididymis, Oxidative Stress, Probiotic, Sperm, *Tsukamurella Inchonensis*

P-43: Magnetic Fe₃O₄ Nanoparticles Modulate The Expression of Pluripotent Genes in Embryos Derived from Vtrified GV Oocytes

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Background: Recently, nanoparticles is widely used to improve the cryopreservation of different cells and tissues special sex cells (sperm and oocyte). According to studies conducted in this area, the vitrification of oocyte alongside with nanoparticles is recognized as a promising technique to cope with the problems which encountered in the freezing process. As respect to deleterious effect of vitrification on oocyte as well as destruction on the embryo development, it can thus be considered whether these nanoparticles are advantageous or not?

Materials and Methods: This study was conducted on the vitrification of immature oocytes with and without magnetic nanoparticles (Fe₃O₄) (Vit and Vit+NP groups) as well as the control group (fresh oocytes). Immature oocytes (GVs) were derived from 6-8 weeks-old NMRI mice and exposed to equilibration medium (7.5%EG plus 7.5%DMSO (v/v)) 5 min, then treated with vitrification solution (VS) (15%DMSO, 15%EG, 0.5 mol/L sucrose) supplemented by nanoparticles (5µgr/ml) 1 min, then they were exposed to warming solutions in three-step sucrose dilution: W1 (1.0 mol/L sucrose) 370c, W2 (0.5 mol/L sucrose) and W3 (0.25 mol/L sucrose) RT for 1, 3 and 3 min. In the following, *in vitro* maturation (IVM) of germinal vesicle oocytes (GV) as well as *in vitro* fertilization (IVF) of obtained mature oocytes were performed after warming in order to achieve 2PN embryos. In 2PN stage we studied pluripotency genes (Oct-4, Nanog, Sox-2 and Cdx2) were amplified using qRT-Realtime PCR. Housekeeping gene for normalization of mRNAs was GAPDH. Expression was calculated using equation; Expression= $(2^{-\Delta\Delta Ct})$.

Results: The expression levels of pluripotent genes, Oct4 and Sox-2 were significantly increased in 2PN embryos derived from vitrified oocytes comparing with non-vitrified embryos but Cdx2 was decreased analytically in Vit+NP group. Nanog didn't has any signal in 2PN embryos. Based on scientific documents this increase does not optimum for embryo development. But after adding 5µgr/ml magnetic Fe₃O₄ nanoparticles to vitrification solution, the expression levels of pluripotent genes (Oct-4 and Sox-2) returned to the natural level.

Conclusion: Using of magnetic Fe₃O₄ nanoparticles to vitrification solution could modulates the expression levels of pluripotent genes (Oct-4 and Sox-2) in pronuclear stage mouse embryos.

Keywords: Oocyte Vitrification, Fe₃O₄ Nanoparticles, Embryo, Pluripotency

P-44: The Comparative Survey of Antioxidant Compositions on MDA, TAC and SOD of Ram Epididymal Sperm

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Background: The anti-oxidant system has been described as a defense functioning mechanism against lipid peroxidation in semen, and is important in maintaining sperm motility and viability. Mammalian sperm membranes incorporate many unsaturated fatty acids and are susceptible to lipid peroxidation (LPO) in the presence of reactive oxygen species (ROS), leading to decreased sperm quality. Ram sperm are subjected to extreme oxidative stress during their preservation in medium. Lipid peroxidation was measured in terms of malondialdehyde (MDA), total antioxidant capacity (TAC) and superoxide dismutase (SOD) levels in semen.

Materials and Methods: In this study, at overall, sperms were collected from 20 pairs of ram testicles. Collection was carried out in slaughtered animals. Sample processing was performed in a walk-in fridge (5°C) immediately after its arrival to laboratory. Then tail of epididymis were fixed between two fingers. Sperm were collected performing several incisions in the caudal epididymis with a surgical blade. Five levels of cysteine (1-2-4-8-10 mM), 4 levels of L-carnitine (2-4-8-10), 4 levels of Glutathione were added into human tubal fluid containing sperms (40×10⁶ sperm/ml), with 10%, bovine serum albumin and were kept for 12 hours at 5°C then at 1 and 12 hrs, the amount of Total anti-oxidant capacity (TAC), Superoxide dismutase (SOD) and Malondialdehyde (MDA) were evaluated with spectrophotometry.

Results: Data were analyzed using SPSS 25 statistical package. Multiple comparisons by post-hoc test such as tukey and tamhane were performed to determine statistical differences among groups. Results were considered significant at P<0.05 for all tests. The results showed that Glutathione at 2-8-10 Mm levels in contrast of control had the best effect on TAC increases and reduction of MDA.

Conclusion: Between amino acids that as antioxidant added to culture medium contain of ram epididymal sperm cells, glutathione had the best effect on sperm TAC and MDA levels during 12 hrs storage at 5°C

Keywords: MDA, TAC, SOD, Ram, Sperm

P-45: The Effect of Cysteine on Kinetic Patterns of Ram Epididymal Sperm during Preservation in Human Tubal Fluid Culture Medium at 5°C

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Background: Ram sperm have a higher polyunsaturated/saturated fatty acids ratio and a lower cholesterol/phospholipid molar ratio than other species, which renders the sperm much more vulnerable to oxidative damage caused by reactive oxygen species (ROS). Oxidative stress subsequently leads to impaired cell function which results in losses of sperm motility, morphological integrity and fertilizing capability, and the induction of sperm apoptosis. The antioxidant capacity of sperm, due to the small cytoplasmic component to scavenge oxidants, is limited. Thus, mammalian spermatozoa may, however, be insufficient in

counteracting the damaging effects of ROS and lipid peroxidation. Additives that act as antioxidative properties reported to reduce the impact of ROS-induced and cold shock damages. Thiol compounds such as cysteine penetrate the cell membrane easily, for the intracellular glutathione biosynthesis both *in vitro* and *in vivo*, has been shown to improve motility of post thawed bull, ram and goat sperm, and to maintain the viability of boar sperm during liquid storage at 5°C. To our knowledge, there is not any study which investigated the role of the cysteine to improve kinetic pattern of ram epididymal sperm in HTF medium.

Materials and Methods: To evaluate the effect of cysteine on ram epididymal sperm kinetic patterns sperms were collected from 15 pairs of mature ram testicles. Collection was carried out in slaughtered animals. After its arrival to laboratory, tail of epididymis were fixed between two fingers. Sperm were collected performing several incisions in the caudal epididymis with a surgical blade. Five levels of cysteine (1-2-4-8-10 mM) were added into human tubal fluid containing sperms (40×10⁶ sperm/ml), with 10%, bovine serum albumin and were kept for 36 hours at 5°C. Sperm motility was examined at 1, 12, 24, 36 hours after kept in 5°C refrigerators with Computer assisted semen analyses (CASA).

Results: The study was repeated three times. The results were expressed as mean ± SEM. Means were analyzed using a one-way analysis of variance, followed by tukey and tamhane post-hoc tests to determine significant differences in all the CASA parameters between all groups using the SPSS version 25. The results showed that cysteine has beneficial effect on sperm quality that evaluated by CASA. The patterns such as progressive motility (%) and velocity (µm/s) of ram epididymal sperm in low levels of cysteine meaningful was better than control groups during liquid storage (P<0.05).

Conclusion: In conclusion adding of low levels cysteine to HTF medium significantly improved the motility patterns and enhanced viability of ram epididymal sperm till 36 hours storage at 5°C.

Keywords: CASA, Ram, Cysteine, Epididymal Sperm

P-46: The Effect of Hydroalcoholic Extract of *Matricaria Chamomilla* on Histological Changes of Testis and Gonadotropins of Rats Treated with Formaldehyde Afrigan L¹, Jafari Anarkooli I¹, Sohrabi D¹, Abdanipour A¹, Yazdinezhad A², Arianmanesh M¹

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Background: Formaldehyde (HCOH) is a colorless gas is used in chemical, textile and cosmetics industries. Formaldehyde is able to produce reactive oxygen species (ROS), damaging testis tissue. Then in present study, the effect of chamomilla extract on the histological changes of testis and gonadotropins was studied in rats treated with formaldehyde.

Materials and Methods: 48 male wistar rats were divided into six groups: 1. Formaldehyde group, 2. Formaldehyde and chamomilla (200 mg/kg), 3. Formaldehyde and chamomilla (500 mg/kg), 4. Chamomilla (200 mg/kg), 5. chamomilla (500 mg/kg), and 6. Control (normal saline 10 mg/kg). Groups receiving formaldehyde was injected 10 mg/kg formaldehyde. All injections were performed intraperitoneally for 30 days (daily). After treatment, rats were killed then, histological changes of

testis, sperm parameters, apoptosis of germ cells, sexual hormones and gonadosomatic index (GSI) were investigated.

Results: Count, motility and viability of sperms and also concentration of testosterone and LH increased significantly ($P \leq 0.05$) in groups treated with formaldehyde and chamomilla compared to formaldehyde group. Apoptotic rate increased significantly ($P \leq 0.05$) in formaldehyde group in comparison with group treated with formaldehyde and chamomilla (500 mg/kg). Also, Miller and Johnson indices in groups treated with formaldehyde and chamomilla elevated significantly ($P \leq 0.05$) compared with formaldehyde group.

Conclusion: Data of this study shown that the extract of *Matricaria Chamomilla* particularly in dose of 500 mg/kg is able to reduce the adverse effects of formaldehyde on the reproductive system of rats.

Keywords: Formaldehyde, *Matricaria Chamomilla*, Testis, Sperm Parameters

P-47: Effects of *In Vitro* Thermal Stress on The Motility of Ovine Epididymal Spermatozoa and The Protective Effect of β -Mercaptoethanol as An Antioxidant

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Background: It has been shown that increased testicular temperature in rams led to decreased sperm count, increased sperm abnormalities and changed seminal plasma proteome. Considering thermal stress impacts on the male fertility and sperm physiology, as well as importance of epididymal spermatozoa as an option for genetic preservation, this study was performed to investigate the effects of thermal stress on the mature epididymal spermatozoa of rams. Besides, due to the increasing evidences indicating the role of excessive ROS production in the thermal stress pathology, the influence of β -mercaptoethanol as an antioxidant also was evaluated.

Materials and Methods: Spermatozoa were recovered from the epididymides of slaughtered rams testes. Aliquots of spermatozoa were incubated at three different temperatures: scrotal temperature (32°C), body temperature (39°C) and heat stressed body temperature (41°C). Along with each antioxidant-free sperm aliquot, an antioxidant-containing aliquot (1 mM β -mercaptoethanol) was incubated at the intended temperature. Assessment of motility parameters was carried out by using computer-assisted sperm analysis system. The factorial ANOVA models were used to determine the statistical differences among the main effects of the incubation temperatures, antioxidant presence and their interactions (temperature \times antioxidant).

Results: Results showed that both the incubation temperatures and antioxidant affected the majority of the motion parameters of spermatozoa ($P < 0.05$). However, the interaction of main effects was not significant in this respect. Regarding incubation temperature, incubation at 41°C had greater detrimental effect on the motility parameters than other temperatures. Presence of antioxidant during incubation period led to the neutralization of the detrimental effects of thermal stress. Presence of antioxidant during the incubation period maintained the motility parameters at the level of Fresh group.

Conclusion: This study showed that ovine epididymal spermatozoa are sensitive to *in vitro* thermal stress and it seems that

this sensitivity is partly related to oxidative stress.

Keywords: Ram Spermatozoa, Thermal stress, Motility, Antioxidant

P-48: The Effect of L-Carnitine on Histomorphology Characteristics of Uterus in Mice with Polycystic Ovary

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Background: Polycystic ovary (PCO) syndrome is the most common endocrine disorder in women of reproductive age that cause infertility and impression in the uterus. Carnitine plays essential roles in energy production, oxidative stress and glucose metabolism. The aim of this study was to investigate the effect of L-carnitine on the histomorphology characteristics of the uterus in mice with polycystic ovary.

Materials and Methods: In the first phase of this study, the mice were divided into two groups; intact mice (I, 12 mice), mice with polycystic ovary (PCO, 12 mice). For induction of polycystic ovary, immature female mice were subjected to daily injections of testosterone enanthate (1mg / 100gr) for 4 weeks under subcutaneous injection in the back of the neck. To verify the induction of polycystic ovary, the observation of the Estrous cycle was conducted until the end of the trial period. In the second phase of the study, each initial group was further divided into two treatment groups; Intact mice (I, 6 mice), Intact receiving L-carnitine (IC, 6 mice), polycystic ovary (PCO, 6 mice) and polycystic ovary receiving L-carnitine (PCOC, 6 mice). L-carnitine was injected intraperitoneally 250 mg/kg for 4 weeks. At the end of the treatment, the mice were sacrificed by cardiac puncture. Blood samples were collected, centrifuged and plasma samples were frozen and stored until hormone analysis. After dissecting, the uterus was isolated for histomorphologic examination. Statistical analysis was performed using one way analysis of variance (ANOVA). Test values with a $P < 0.05$ were considered significantly different.

Results: The results showed that induction of polycystic ovary in immature female mice increased body weight, decreased endometrial, and uterine muscle thickness, decreased the number of glands and total uterine diameter. In contrast, L-carnitine decreased the excess body weight caused by polycystic ovary and increased endometrial and uterine muscle thickness and uterus size, and ultimately increased uterine mass compared to the polycystic group. Induction of PCO increased the concentration of testosterone compared to the control group, but L-carnitine had no effect on testosterone concentration.

Conclusion: It can be concluded that L-carnitine can reduce the complications of the polycystic ovaries of PCO mice, restore their normal ovarian-uterine cycles and markedly reduce the complications of the polycystic ovaries in the maternal reproductive system. These findings showed that L-carnitine could stimulate ovulation and ultimately increase fertility in our PCO model mice.

Keywords: L-carnitine, Polycystic Ovary, Uterus, Mice, Testosterone

P-49: Influence of Frozen Bovine Ampullary-Oviductal Epithelial Monolayer Cell Co-Culturing during *In Vitro* Maturation on Embryo Development and Vitrification

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Background: Co-culturing of bovine embryos with fresh oviductal-epithelial cells, albeit may improve *in vitro* production of bovine embryos, is time-consuming and not reliable. Therefore, the following study was designed to examine the influence of co-culturing frozen-thawed bovine ampullary cells on oocyte *in vitro* maturation (IVM) and subsequently *in vitro* development of bovine embryos.

Materials and Methods: Five days prior to using cell monolayer in IVM, a batch of frozen ampullary cells was thawed in 37°C sterile water and added to culture media (TCM-199, 15% FCS and 40 µg/ml gentamicin). The cells were then centrifuged for five minutes at 400g to omit DMSO. The retrieved cells were then re-suspended in warm culture media and seeded into 4-well NUNC plates with a concentration of 1×10^6 cell/ml. The media was refreshed every 48h and changed to maturation media one day before co-culturing with the immature bovine oocytes. The oocytes (n=469) were then co-cultured under two different conditions (5 replicates). In control group, the oocytes (n=232) were cultured for 24h in a free cell maturation media (TCM-199 (Sigma, M4530), 10% FCS, 50 µg/ml gentamicin (Sigma, G1272), 5 IU/ml highly purified hCG (Karma, Pharmatech GmbH, Germany), 0.1 IU/ml recombinant human FSH (Follitrope, LG Life Sciences, South Korea), and 10 ng/ml epidermal growth factor (E4127)). In the treatment group, the oocytes (n=237) were cultured in maturation media for 18h and were then transferred into a media containing bovine ampullary oviductal epithelial cell (BAOEC) for a further 6h of incubation. After 24h of incubation, the matured oocytes in both groups were transferred into dishes containing *in vitro* fertilization media and capacitated sperm (concentration of 1×10^6 sperm/ml). About 18-20h after insemination, presumptive zygotes were denuded and transferred into the plates containing synthetic oviductal fluid media. The culture media was refreshed every 48h.

Results: Cleavage rate (48h post insemination), blastocyst rate (day 8 post-insemination), and blastocyst cryotolerance (assessed by vitrify/thawing early and mid-stage blastocysts) were recorded. The data were analyzed using logistic regression model through PROC GENMOD in SAS. The cleavage rate (79.7 and 80.1% for control and treatment groups, respectively) was not affected by the treatment whereas blastocyst rate (number of blastocytes/number of oocytes) in the treatment group (29.3%) was significantly ($P < 0.05$) higher than that in the control group (24.5%). The treatment group showed higher embryo survival than the control group at both 24h (odds ratio \pm (0.95% CI)=4.4 \pm (1.23-15.8) for treatment vs. control group) and 48h (odds ratio \pm (0.95% CI)=4.2 \pm (1.15-15.37) for treatment vs. control group) after embryo thawing.

Conclusion: In conclusion, adding ampullary-epithelial cell monolayer to oocyte maturation media in the last 6h of IVM can improved quantity and quality of IVP embryos.

Keywords: Ampullary-Oviductal Cells, Oocyte Maturation, Blastocysts, Bovine

P-50: Antioxidant Effects of Royal Jelly Ameliorate Nico-

tine-Induced Testicular Injuries in Mice

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Background: Nicotine (NIC), a pharmacologically active alkaloid, is the most active substance in tobacco causing cell division disorder, apoptosis and malformation. This study evaluated the possible protective effect of royal jelly (RJ) on testicular histological damages and antioxidant status in NIC-exposed male mice.

Materials and Methods: 36 male BALB/c mice were randomly divided into six groups (n=6). Group 1 received normal saline, group 2 received 100 mg/kgBW/day RJ, groups 3 and 4 received NIC at doses of 0.50 and 1.00 mg/kgBW/day, respectively and groups 5 and 6 received NIC at doses of 0.50 and 1.00 mg/kg BW/day respectively plus RJ. Following 35 days, all animals were sacrificed and testicular tissues were used for biochemical (MDA, TAC and CAT) examinations and histopathological (TDI, SPI, MI and SCI) studies.

Results: Our observations showed that NIC led to remarkable increased MDA concentration and reduced the levels of TAC and CAT in testicular tissue versus control group. Besides, NIC caused remarkable diminishing in TDI, SPI, MI and SCI indices in comparison with control group. While RJ co-administration could improve all above-mentioned alterations versus NIC receiving groups.

Conclusion: Data from the current study suggest that RJ has a potential repro-protective action against NIC-induced histological abnormality and oxidative stress in mice.

Keywords: Nicotine, Royal Jelly, Oxidative Stress, Histology, Testis

P-51: The Effect of Erythropoietin on Learning and Memory in Rat Model of Intrauterine Growth Restriction

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Background: Intrauterine growth restriction (IUGR) leads to abnormalities in the foetal central nervous system, till apoptosis became in hippocampal and cortical cells. The goal of this research is investigating the effects of erythropoietin (EPO) on hippocampal cell density, learning, and memory in the rat model of IUGR.

Materials and Methods: In order to induction of IUGR, pregnant Wistar rats were under 50 % restricted diet from 14 embryonic days (ED). From 12 ED, EPO- treated rats were received 500, 1000 and 2000 U/kg EPO, subcutaneously. After the birth of pups, morphological evaluations were checked. Then, in 30 postnatal days after cardiac perfusion, histopathologic and stereologic studies of hippocampus were assessed. The working and passive avoidance memories were analyzed by Y-maze and shuttle box tests.

Results: The cell density in CA1, CA2, CA3 and dentate gyrus of hippocampus in IUGR rats was reduced significantly compared to the controls. In addition restricted diet leads to decrease in working and avoidance memories. On the other hand,

EPO was ameliorated hippocampal cell damages and increased working and passive avoidance memories in treated rats.

Conclusion: The findings suggest that restricted diet during pregnancy can cause hippocampal cell deficits in rats. This is likely due to decreased learning and memories. The results also suggest that the neuroprotective effect of EPO is likely to improve hippocampal cell damages in IUGR rat model.

Keywords: Hippocampus, Intrauterine Growth Restriction, Memory, Erythropoietin

P-52: 6-Gingerol Improves Human Freezing Thawing Process

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Background: Cryopreservation of human sperm associated with an increase in reactive oxygen species (ROS) which lead to lipid peroxidation of sperm membrane. Natural antioxidants improve the quality and metabolic activity of sperm during the cryopreservation. Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) contains volatile oil rich pungent principles (Shogaols, zingerone, and gingerols) that exhibited substantial antioxidant activities. In this study we evaluated the effect of gingerol as an antioxidant in sperm cryopreservation medium on different sperm parameters during the freezing thawing process.

Materials and Methods: Semen were collected from 20 normospermic men referring to the Royan Institute and divided into three groups; Fresh group, control freezing group and freezing with 6-gingerol (final concentration of 10 μ M) group. Finally, sperm motility the levels of malondialdehyde (MDA) and acrosome intact were evaluated in all groups.

Results: Evaluation of sperm motility was performed with computer-assisted-semen analysis (CASA). Total motility and progressive motility was significantly lower in freezing group (29.90% \pm 2.13% and 30.03% \pm 2.82 resp) compared to fresh group (61.27% \pm 2.95). However, MDA levels in the freezing group were higher than fresh group (P<0.05). There was no significant difference between freezing and freezing + Gingerol in sperm motility and MDA levels. Acrosome intact were evaluated by hypo osmotic swelling test (HOST). Acrosome intact in fresh and freezing + Gingerol groups were significantly higher (84.45% \pm 1.94% and 63.50% \pm 1.41% respectively) than freezing group (53.25% \pm 1.51).

Conclusion: Accordingly, we concluded that although Gingerol improved sperm viability, it did not show positive effect on MDA levels and motility. It seems that addition of Gingerol to freezing medium can improve sperm freezing condition.

Keywords: Cryopreservation, Sperm Quality, Gingerol

P-53: Growth of Human Endometrial Cells on Electrospun Polycaprolactone/Polydimethylsiloxane Nanofibrous Scaffold for Uterine Tissue Engineering

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Background: In this study, a novel PCL/PDMS scaffold with improved mechanical properties and controllable porous structure was prepared through electro spinning method. The scaffold biocompatibility was further studied, using human foreskin fibroblast. MTT assay results indicated that human endometrial cells grew well on the PCL/PDMS scaffold with a notable parallel arrangement. Although further studies, such as extracellular matrix production and functional gene expression, are necessary to confirm the proper biocompatibility of the PCL/PDMS scaffolds, the results indicate that the PCL/PDMS scaffold could be a new suitable scaffold for uterine tissue engineering

Materials and Methods: Polycaprolactone (PCL) and polydimethylsiloxane vinylterminated were purchased from Sigma-Aldrich® and Trifluoroacetic acid (TFA) were purchased from Merck® companies. Endometrial cells were extracted from normal human uterine tissue samples

Results: Endometrium cell were cultured in DMEM. a surface phenotype (CD90+, CD105+, CD146+, and CD45) distinguishing them from mesenchymal stem cells (MSCs). After sterilization under UV light for 2 h, the electrospun fibers were placed in 24-well plates and were seeded with endometrial cells at a density of 10,000 cells/well. The cells were incubated in a humidified standard incubator at 37 °C with 5% CO₂. Cells were harvested at days 3, 7, and 10 for growth, phenotypical evaluation and MTT test. By triplicate assay the activity and survival of the cells on nanofibers were calculated as a percentage of samples to control ratio.

Conclusion: The random, aligned scaffolds were developed by the electrospinning technique, and their morphological microstructures were investigated by SEM. SEM micrographs of the scaffolds were obtained before seeding of the cells. The human fibroblasts showed a well growth and replication on the scaffolds. The endometrial cells on all four types of scaffolds were found to spread well and the cells were grown on the scaffold. Also, significant difference in cell growth and replication on the scaffold and control samples indicates the lack of toxicity of the scaffold for the scaffold adaptation. These findings could be used in endometrial tissue engineering as the first step for uterine tissue engineering. We planned to evaluate a fertilized egg response on this novel scaffold.

Keywords: Endometrium, Nano Fiber, Polycaprolactone/Polydimethylsiloxane, Scaffold, Tissue Engineering

P-54: Clinical Feature of Bacteriospermia in Men Included in The *In Vitro* Fertilization or Intracytoplasmic Sperm Injection Program

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Background: Approximately 60% of patients under treatment of assisted reproductive technology (ART) had suffered local

inflammation/infection. The bacterial infections may impair reproductive function. Therefore, the aim of this study was to investigate the effect of bacterial infections in abnormal chromatin packaging of spermatozoa and fertilization rate in *in vitro* fertilization (IVF) cycles in comparison to intracytoplasmic sperm injection (ICSI) cycles.

Materials and Methods: To assess whether bacterial infections can influence ICSI or IVF outcomes in terms of fertilization, a study was conducted between October 2017 to February 2018. A total 57 ICSI cycles and 22 IVF cycles for exclusively male infertility factors were included. Microbiological analysis of semen samples was performed within 1 hour after ejaculation. Semen samples were prepared and used to fertilize the retrieved oocytes. Fertilization outcome was assessed almost from 18-20 hours after ICSI/IVF process, and evaluated among groups.

Results: Overall, 40.4% of studied samples were infected with bacteriospermia (such as *E. Coli*, *Staphylococcus saprophyticus*, etc.). In IVF group, the presence of bacterial infections showed significantly decrease in successful fertilization as compared to group without bacterial infection (86.36% vs 90.22%, respectively, $P < 0.05$). While fertilization rate was the same between samples with and without bacterial infections in ICSI group (63.14% vs 63.55%, respectively, $P > 0.05$).

Conclusion: The presence of bacteriospermia in the sperms used in IVF cycles with failed fertilization creates the possibility that some bacterial agents may connect to spermatozoa and influence its parameter such as motility and fertilization potential. Therefore, the rate of successful fertilization may be affected by bacterial infections. Hence, further ultra-structural studies are needed.

Keywords: Bacteriospermia, Fertilization Rate, Motility, IVF, ICSI

P-55: Human Sperm Associated Antigens (SPAGs) Down-regulation Post Freezing

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Background: Human sperm membrane protein or sperm associated antigens (SPAG) includes 18 types of proteins that some of these SPAGs play a significant role in fertility. The rapid freezing of sperm, which is thought to be a tool for ART, may disrupt the expression of these SPAGs. The aim of this study was to evaluate the effects of normal human sperm freezing on the gene expression level of these very important antigens. But the question is, what is the optimal recovery time point after freeze-thawing human spermatozoa, which allows the cells to resume their gene expression?

Materials and Methods: The semen samples were collected from 4 normospermic individuals. The aliquots were divided into three equal volumes including one fresh and two freeze groups (with recovery time of 1 hour and 2 hours separately).

Before cryopreservation, sperms were selected with Density Gradient Centrifugation (DGC) procedure. In freezing, sperms samples were mixed with spermfreeze[®] solution as cryoprotectant (1:0.7) for 10 minutes. Then the mixture was loaded into cryotube and frozen with rapid freezing procedure. After 3 days storage times, the specimen were thawed in tap water for 5 min and then washed with HTF (Human Tubal Fluid). Thawed samples were incubated for 1 and 2 hours at recovery time. Evaluation of SPAGs gene expression in fresh and freeze-thawed sperm samples were performed by real-time PCR technique.

Results: Our findings showed there were no significant differences between two different recovery time points (2 hours and 1 hour), although SPAGs genes expression level were increased after 2 hours post freezing recovery time in comparison to 1 hour except SPAG 3, 4, 5, 7 and 9. Moreover the expression level of SPAGs 1, 5, 6, 7, 8, 12 and 18 were significantly decreased compared to the fresh sample assessed in 2 hours post rapid sperm freezing.

Conclusion: In present study, we showed that cryopreservation procedure could negatively affect on some of SPAGs genes expression in human spermatozoa. Also for the evaluation of freezing effects on sperm, 2 hours is better to assess than 1 hour post freezing.

Keywords: Human Sperm, Sperm Associated Antigens, SPAG, Freeze, Real-Time PCR

P-56: Bioengineered Human Ovarian ECM Reconstructs GDF-9 Positive Follicles

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Background: Since access to a practical way for achieving the mature follicles from cryopreserved or native ovarian tissues special in huge animals is difficult, tissue engineering is very promising to provide artificial folliculogenesis. Decellularization is a process to remove cellular materials except the organ skeleton and extracellular elements (ECE) to make a bio-scaffolds. In the present study, the effect of sodium hydroxide (NaOH) has been investigated in decellularization of human ovarian tissue applied to follicular reconstruct.

Materials and Methods: Human ovarian pieces were distributed in two groups consisted of control and NaOH treated (0.5 M). Qualitative histological evaluations, quantitative assessments (DNA, total collagen and glycosaminoglycan contents), immunohistochemistry (IHC) staining for Laminin, Fibronectin and Collagen I, cell viability and electron microscopic assay

were performed. Finally, human NaOH treated scaffolds along with mouse ovarian cells as hybrid artificial ovary (hAO) were allo-transplanted to ovariectomized mice. H & E technique and IHC for GDF-9 was performed on ovarian tissue decellularized one month after transplantation.

Results: Histological studies and quantitative evaluations confirmed the successful decellularization and presence of key factors in ovarian scaffolds. Also, toxicity test showed NaOH treated scaffolds well maintained the survivability of fibroblast cells. Moreover, spherical associations with cuboidal cells in transplanted scaffolds were observed which GDF-9 expression was confirmed follicular reconstruction.

Conclusion: NaOH is an appropriate material for eliminating the ovarian cells and supporting the new implanted ovarian cells to follicular reconstruction which can be a valuable finding in tissue bioengineering research to provide a hybrid artificial ovary.

Keywords: Ovary, Decellularization, NaOH, Bioscaffold, Follicular Reconstruction

P-57: Full Maturation and Ovarian Follicular Growth in 3D Auto-Transplantation of Mouse Preantral Follicles Applying Wharton's Jelly Hydrogel

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Background: Applying common fertility treatment such as frozen ovarian tissue transplantation due to return of malignant cells is not efficient in treating all cases of infertility. Therefore, invitro follicle culture, decellularization of tissues and specific reproductive organs to use the extracellular matrix is an appropriate replacement for frozen ovarian tissue transplantation. The purpose of this study was to investigate the potential of stability, angiogenesis, reconstruction and regeneration of human Wharton's jelly hydrogel in 3D auto-transplantation of mouse preantral follicles.

Materials and Methods: 35 NMRI mice 6 to 8 weeks old were divided into 4 groups, control group 1: Intact mouse (INT), control group 2: Gonadectomized mouse (GON), experimental group 1: Whole ovary transplant (WOT), the experimental group 2: Follicle hydrogel transplant (FHT). Both ovaries of the WOT, FHT and GON mice were resected from body under general anesthesia. In the WOT group, the right ovary was implanted inside the peritoneum of the mouse. In the GON group, the ovaries were removed and the mice were kept for 2 weeks to assess the serum level of follicle stimulating hormone (FSH) and estradiol hormone levels. In the FHT group, both ovaries were placed in 50 µl drops of α-MEM medium and preantral follicles were isolated, encapsulated and transplanted for 2 weeks.

Results: After two weeks of transplantation, it was determined that human Wharton's jelly hydrogel has been able to restart folliculogenesis, support the growth of follicles and their spherical structure. Hormonal evaluation showed that despite the increase of serum FSH level in the FHT group compared to the

GON and INT groups, serum estradiol level increased in FHT group compared to GON group but reduced comparing with the INT and WOT groups there was no significant difference ($P>0.05$). Despite the establishment of a sexual and hormonal cycle in most of the mice, the onset of the estrous cycle was postponed but no significant difference was observed ($P>0.05$). Immunohistochemical evaluation was confirmed the expression of VEGF and CD34 proteins in the cumulus cells around the oocyte, ovarian stroma, corpus luteum, granulosa cells and follicular fluid. These results showed angiogenesis in the FHT group has been occurred without using any external hormone or supporting cells in comparison with control groups.

Conclusion: It can be concluded from this study that human Wharton's jelly hydrogel can support the growth of follicles and restore the cycle of folliculogenesis in the body.

Keywords: Ovary, Preantral Follicle, Hydrogel, Transplantation, Human Wharton's Jelly

P-58: The Study Protective Effect of Vitamin E on Sperm Parameters in Glyphosate Treated Male Rats

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Background: Glyphosate, an N-(phosphono methyl) glycine is a broad spectrum herbicide widely used to eliminate unwanted plants which is responsible for many harmful effects on reproductive health such as endocrine disrupting effect in human embryonic cells, induced sperm abnormalities, decrease in the sperm count and sperm motility. The present study aimed to evaluate the protective effects of vitamin E (vit E) against harmful effects of glyphosate on sperm parameters.

Materials and Methods: 36 adult male rats were randomized into six experimental groups (n=6). group 1 received 0.20 ml normal saline (control), group 2 received 250 mg/kg Glyphosate, group 3 received 500 mg/kg Glyphosate, group 4 received 100 mg/kg vit E, group 5 received 100 mg/kg vit E+250 mg/kg Glyphosate, group 6 received 100 mg/kg vit E +500 mg/kg Glyphosate. All administration were done orally. After 56 days, Changes in sperm parameters were evaluated.

Results: Sperm parameters analyze showed that glyphosate groups had significant reduction of sperm count, motility, viability and significant increase in sperm abnormality compared with control group. Vitamin E co-administration significantly ($P<0.05$) improved harmful effects of glyphosate on mentioned sperm parameters.

Conclusion: Our results demonstrate that vitamin E has protective effect on sperm parameters against the glyphosate negative effects.

Keywords: Glyphosate, Male Rat, Sperm Parameters, Vitamin E

P-59: The Study Protective Role of Spirulina Platensis Against Oxidative Stress Caused by Glyphosate in Male Rats Testicle

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Background: Glyphosate, is a broad spectrum herbicide widely used to eliminate unwanted plants. Glyphosate was toxic for human embryonic cells, induced Sperm abnormalities and oxidative stress. *Spirulina platensis* (S) can reduce oxidative stress, testicular damage, and sperm abnormalities by its potent antioxidant activity. This study was conducted to determine the protective role of *spirulina platensis* against Oxidative stress caused by Glyphosate.

Materials and Methods: 36 adult male rats were randomized into six experimental groups (n=6). group1 received 0.20 ml normal saline (control), group2 received 250 mg/kg Glyphosate, group3 received 500 mg/kg Glyphosate, group4 received 500 mg/kg S, group5 received 500 mg/kg S+250 mg/kg Glyphosate, group6 received 500 mg/kg S+500 mg/kg Glyphosate. All administration were done orally. After 56 days, Malondialdehyde (MDA) values and Total antioxidant capacity (TAOC) were measured by the Chessman-Esterbauer and FRAP method, respectively.

Results: The data indicated that Glyphosate groups had significant reduction of TAOC, and significant increase in MDA values compared with control group. *Spirulina platensis* co-administration significantly (P<0.05) improves the reduction of TAOC caused by glyphosate, it also reduces MDA levels.

Conclusion: *Spirulina platensis* can reduce oxidative stress by its potent antioxidant activity and noticeably can improve adverse effects of glyphosate on male reproduction system.

Keywords: Glyphosate, Male Rat, Oxidative Stress, *Spirulina Platensis*

P-60: Effect of Aqueous Carica Papaya Seed Extract on Human Sperm *In Vitro*

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Background: *Carica papaya* seeds are used in traditional medicine as a male contraceptive. Researchers have investigated its effects in various animals such as dogs, rats and monkeys *in vivo* showing contraceptive effects.

Materials and Methods: *C. papaya* seeds were washed, dried and ground to powder. Then, 5g of this powder were extracted with 200 ml of distilled water heated at 70°C for 72 hours. The extract was subsequently filtered, frozen at -20°C and freeze-dried. Motile human sperm were obtained from 35 healthy donors. Sperm were isolated by means of swim-up and then incubated for 60 minutes in Human Tubular Fluid Medium containing 1% bovine serum albumin with different concentrations (zero [control], 0.025, 0.25, 2.5, 25, 250 and 2500 µg/ml) of the extract. Vitality, various motion parameters, the percentage of sperm producing reactive oxygen species (ROS), mitochondrial membrane potential (MMP), DNA fragmentation, capacitation and acrosome reaction were determined.

Results: Results show that sperm motion parameters such as total motility, VSL, LIN, STR and BCF did not change compared to the control. Whereas, progressive motility, VCL, VAP and the percentage of hyper-activated sperm decreased significantly

(P<0.05). Furthermore, while no difference was observed for the percentages of vitality, ROS-positive sperm, capacitation and acrosome reaction, the extract caused significant changes in the percentages of MMP and DNA-fragmented sperm (P<0.05).

Conclusion: Aqueous *C. papaya* seed extract affects progressive motility, VCL, VAP, hyper-activation, MMP and DNA fragmentation. However, total motility, VSL, LIN, STR and BCF alongside the vitality and ROS were not affected.

Keywords: *Carica Papaya*, Human Sperm, Contraceptive, *in vitro*

P-61: The Effects of Aqueous Carica Papaya Seed Extract on Sertoli and Leydig Cell Lines

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Background: It has been shown that extracts from the seeds can affect testes causing reversible sterility.

Materials and Methods: *C. papaya* seeds were washed, dried and ground to a powder, of which 5g were added to 200 ml of distilled water. This mixture then heated for 72 hours at 70°C. Thereafter, the remaining water containing the seed debris was filtered. The aqueous extract was frozen at -20°C and subsequently freeze-dried to obtain a powder, which was used to make up the following concentrations in Dulbecco's Modified Eagle medium supplemented with 5% fetal bovine serum, 2.5% donor equine serum and 1% penicillin-streptomycin (zero (control), 0.000025, 0.00025, 0.0025, 0.025, 0.25, 2.5, 25, 250 and 2500 µg/ml). TM3 and TM4 cell lines were incubated with all concentrations for 24, 48, 72 and 96 hours in 24-well plates under acute and chronic conditions. TM3 (Leydig) and TM4 (Sertoli) cells were trypsinated and stained with 0.4% trypan blue.

Results: The total number of TM3 cells significantly decreased after 24, 48, 72 and 96 hours of exposure with aqueous *C. papaya* seed extract (P<0.05). Furthermore, the total number of TM3 and TM4 cells did not change after 24 hours of exposure, but were significantly (P<0.05) decreased after 48, 72 and 96 hours. Moreover, viability of TM4 cells did not change after 24, 48, 72 and 96 hours of acute exposure, whereas it decreased significantly (P<0.05) after 48, 72 and 96 hours of chronic exposure.

Conclusion: In conclusion, aqueous *C. papaya* seed extract affected the division of both TM3 and TM4 cells as well as viability of the acute and chronic exposure between 48 and 96 hours.

Keywords: *Carica Papaya*, TM4, TM3, Contraceptive, *In Vitro*

P-62: The Effect of Royal Jelly on Follicular Development in Female Rats

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Background: The aim of present study was to evaluate the effect of RJ on some reproductive parameters in adult female rats.

Materials and Methods: Twenty-eight adult female rats (180-200 gr) were divided into four groups (n=7 /group). Control group received 0.5 ml distilled water intraperitoneally (i.p),

experimental groups received: 100, 200 and 400 mg/kg/body weight doses of RJ daily for 14 days respectively. Animals were sacrificed and ovaries were dissected for histopathologic examination; the serum levels of ovarian hormones were evaluated. The ratio of the ovarian and uterine weight to body weight was calculated. One-way ANOVA was used for data analysis.

Results: The body weight was significantly increased ($P=0.004$) in 100, 200 and 400 mg/kg RJ treated animals. The serum levels of progesterone ($P=0.013$) and estradiol ($P=0.004$) were increased in experimental groups significantly. In addition, histopathological data of ovaries showed a significant increase in the number of mature follicles and the number of corpora lutea ($P=0.007$).

Conclusion: The overall results of the present study provide evidence on the ovarian folliculogenesis effect of RJ in female rat.

Keywords: Royal jelly , Fertility , Ovary , Uterus , Rat

P-63: The Effect of Selenium on Spermatogenesis in Mice Treated with Flonicamid

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Background: The use of pesticides in agriculture is necessary in these days. Pesticides produce free radicals and damage to the reproductive system, hence, antioxidants can be used to improve the adverse effect of pesticides. Antioxidants are substances that reduce or prevent cells damage by neutralizing free radicals. Selenium is essential for much basic physiological reaction to protect the cells from oxidative stress. In this study we studied the effect of Selenium on spermatogenesis process against the Flonicamid pestiside.

Materials and Methods: Accordingly, adult male NMRI mice 10 weeks old divided into 6 groups, group 1: Control group received nothing. group 2: Flonicamide 25 mg / kg, group 3: Flonicamide 25 mg / kg and sodium selenite 0.5 mg / kg, group 4: Flonicamide 50 mg/ kg, group 5: Flonicamide 50 mg/ kg and sodium selenite 0.5 mg / kg and group 6: sodium selenite 0.5 mg / kg. All groups were injected intraperitoneally daily for 21 days. After injection, the mice were sacrificed by displacement of the cervical vertebrae. Testes and caudal epididym were removed for histomorphological and sperm parameters studies.

Results: Histological studies showed that Flonicamide significantly reduced the number of sertoli cells, spermatogonia, primary spermatocytes, round and elongated spermatids, and in the sperm parameters studies, decreased count, viability, motility of sperm and increased sperm abnormality compared to the control group and this damage was sharp in high dose.

Conclusion: The results showed that sodium selenite whit flonicamide improved all tissue and sperm parameters damage, So, sodium selenite can reduce the negative effects of Flonicamide on spermatogenesis and improve the quality of sperm and can introduce as a good antioxidant for reduce the adverse effect of pesticide specially Flonicamid.

Keywords: Flonicamid, Sodium Selenite, Pesticide, Spermatogenesis, Mice

P-64: Weighted Gene CO-Expression Network Analysis Revealed Dynamic Transcriptome Alteration in Human Preimplantation Embryonic Cells

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Background: Preimplantation embryo in human is divided into several stages. These stages comes with important processes affecting next levels of embryo development. It has been revealed that each stage of preimplantation embryo has its specific transcriptome. As a high throughput data, a way to analyze transcriptome is network based studied in the systems level. Weighted Gene Co-expression Network Analysis (WGCNA) is a reliable and broadly applied network analyses that uses transcriptome data to detect modules of highly correlated expressed genes. Here, we have applied WGCNA to detect module of genes which are related to each stage of preimplantation embryo. We also analyzed transcription factors (TFs) related to each stage to see transcription regulatory alterations during preimplantation embryo. Additionally, by detecting coordinates of members of significantly related modules to the stages we looked for potential common motifs in their upstream region.

Materials and Methods: We collected a RNA-seq dataset (GSE36552) contains several samples for Oocyte, Zygote, 2 cell, 4 cell, and 8 cell. Matrix of RPKM values were processed to remove unfavorable genes to reduce noise and bias. Then, WGCNA analyses were performed using R programming and annotation of stage-related modules were considered applying DAVID database. Parallel plot of expression values of the module members were detected to check the trend of gene expression during preimplantation. All TFs involved in preimplantation embryo stages were detect comparing the matrix with all TFs of human. Coordinate of the module members were obtain using genome table of UCSC database and motifs in -1500 region were analyzed by RSAT database.

Results: After processing of early matrix, we prepared a matrix contain 10850 genes. We detected several modules related to each stages. Annotation of modules detected that transcription, cell division, and cell adhesion are main biological processes in all stages. Parallel plots showed that almost all the genes in the modules are upregulated in zygote to 4 cell stages and down-regulated in 8 cell stage. Clustering of TFs by correlation grouped them into two subclasses that are differ in expression pattern. They grouped samples into 3 different clusters including zygote, oocyte and 2 cell in a cluster, 4 cell and 8 cell in separate clusters, as well. TFs that control the clustered TFs were predicted and grouped into two clearly different clusters. The motif discovery analyses also detected some specific motifs at upstream region of the module members.

Conclusion: Our analysis have detected some specific TFs and motifs for preimplantation embryo in human. These results may shed light on embryo development knowledge and may be helpful for future studies.

Keywords: Embryo, RNA-Seq, Transcription Factors, WGCNA

P-65: The *In Vitro* Effects of Calcitriol on Seminal Traits in Chukar Partridge (*Alectoris chukar*) Breeders

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Background: There is generally a decrease in the quality of *in vitro* preserved spermatozoa which limits the use of artificial insemination in many species. This study was aimed at possible increases in the quality of *in vitro* preserved partridge spermatozoa when the semen extender was supplemented with calcitriol. **Materials and Methods:** Sixty male partridges were habituated to semen collection by abdominal massage. The seminal concentration of calcitriol was initially quantified (12 mg/mL). Ejaculates from males were pooled and extended (1 to 5) in Sexton's diluent containing 0, 24, 48, 96 or 192 µg calcitriol per milliliter. Twelve sub-samples for each treatment group were kept at 4-5°C for 48 hours. The percentage of motile sperm, live sperm (eosin-nigrosin staining), and concentration of thiobarbituric acid reactive species (TBARS) were determined in triplicate. The data were analyzed by the mixed procedure of STATA software.

Results: The percentage of motile sperm, live sperm, and seminal TBARS were affected by calcitriol ($P < 0.05$). A significant interaction effect of calcitriol, storage duration, and storage temperature was also detected.

Conclusion: Supplementation of the diluent with 196 µg calcitriol per milliliter resulted in the highest sperm motility at 4°C. The same treatment group recorded the highest sperm viability and lowest seminal TBARS as well. Supplementing the diluent with calcitriol had beneficial effects on the Chukar spermatozoa; however, fertility rate of the sperm extended in calcitriol-supplemented diluent needs to be determined before the procedure could be recommended for probable use in artificial insemination programs.

Keywords: Oxidative stress, Partridge, Semen, Sperm, Vitamin D

P-66: Features of Chukar Partridge (Alectoris Chukar) Sperm: Alterations at Different Holding Times and Temperatures

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Background: The paucity of information on seminal characteristics and their alteration at different holding temperature and holding time led to the present study to provide more data required for efficient *in vitro* storage of partridge semen.

Materials and Methods: Sixty male Chukar partridges were habituated to abdominal massage for semen collection. The ejaculates from males were pooled and extended (1 to 5) in Sexton's diluent. The samples were kept at 4-5°C or 19-24°C holding temperature for 4, 24, or 48 hours. The percentage of motile sperm, live sperm (eosin-nigrosin staining), and concentration of thiobarbituric acid reactive species (TBARS) were determined in triplicate. The data were analyzed by the mixed procedure of STATA software.

Results: Data showed that the percentage of sperm motility and sperm viability was higher (81.7 and 91.6%, respectively) at lower holding temperature and shorter duration of storage time. Also, a lower value of seminal TBARS was recorded at the low

holding temperature and shorter storage time.

Conclusion: The percentage of sperm motility and viability was higher and the concentration of TBARS was lower at the lowest temperature and storage time applied in the current study. Further improvement in the quality of the extended Chukar semen is needed before *in vitro* preservation over long periods can be recommended for artificial insemination.

Keywords: Alectoris Chukar, Partridge, Sperm, Storage, Temperature

P-67: The Effect of Human Seminal Plasma on Induced Polycystic Ovary by Testosterone Enanthate in Mice

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Background: Polycystic ovary syndrome is the most common cause of infertility in women of reproductive age. The lack of ovulation is one of the most common symptoms of this disease. The main objective of this study was to investigate the effect of human seminal plasma on histomorphology of polycystic ovary in mice.

Materials and Methods: In this study, the mice were divided into 3 groups; control, PCO and PCO/seminal plasma. For induction of polycystic ovary, testosterone enanthate 1 mg/100g was injected in immature mice for 4 weeks. To ensure polycystic ovary induction, the vaginal smear of the mice examined. After induction of polycystic ovary, seminal plasma group received PMSG hormone at a concentration of 10 IU and 48 hours later human seminal plasma 0.1ml, i.p, in a single dose was injected. Body weight and ovarian diameter were measured. Also the estrous cycle, changes in histomorphology of the ovary, characteristics of ovarian tissue, the number of follicles and the number of oocytes in oviduct were investigated.

Results: In our results, seminal Plasma did not affect the weight of the mice. Also increased the diameter of the ovary in PCO/Seminal plasma group compared to the PCO group. In PCO/Seminal plasma group, the Estrous cycle changed from the diestrous phase to the estrus phase. seminal plasma decreased hyperthecosis, the number of disrupted and pyknotic granulosa cells and hyper vascularization, also increased the number of luteinization of granulosa cells. the average of antral follicles and corpus luteum significantly increased in the PCO/Seminal plasma group compared to the PCO group.

Conclusion: Seminal plasma induces ovulation in the polycystic ovary.

Keywords: Polycystic Ovary, Human Seminal Plasma, Testosterone, PMSG Hormone

P-68: The Effect of Human Seminal Plasma, hCG and Metformin on Induced Polycystic Ovary in Mice

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Background: Polycystic ovary syndrome is the most common endocrine disorder and the most important cause of infertility due to anovulatory in women. One of the ways to cure this dis-

ease is to induce the ovulation. The main objective of this study was to evaluate the effects of human seminal plasma, hCG and metformin on polycystic ovary syndrome in mice.

Materials and Methods: In this study, testosterone enanthate 1 mg/100g was injected into immature mice for induction of polycystic ovary for 4 weeks. Then the mice were divided into 6 groups; control group: received nothing. seminal plasma group: received PMSG hormone at a concentration of 10 IU and 48 hours later received human seminal plasma 0.1 ml, i.p, in a single dose, hCG group: received PMSG hormone at a concentration of 10 IU, i.p, in a single dose. metformin group: received metformin 250 mg / kg, i.p, for 2 weeks. Metformin/ seminal plasma group: received metformin 250 mg / kg for 2 weeks, then injected PMSG hormone with a concentration of 10 IU and 48 hours later received human seminal plasma 0.1 ml, i.p, in a single dose, metformin/hCG group: received metformin 250 mg / kg for 2 weeks, then injected PMSG hormone with a concentration of 10 IU and 48 hours later received hCG at a concentration of 10 IU, i.p, in a single dose. Body weight, ovarian diameter were measured and the histomorphology of the ovary, characteristics of ovarian tissue, also the estrous cycle, the number of follicles and the number of oocytes in oviduct were investigated.

Results: The results of the study showed that seminal plasma and hCG did not affect the mice weight, increased the number of antral follicles and corpus luteum, and changed the estrous cycle from the diestrous phase to estrus phase. Seminal plasma affected the quality of the ovary, and significantly increased the diameter of the ovary. Also hCG increased the number of preantral follicles. Metformin reduced the mice weight, increased the diameter of the ovary, increased the number of antral follicles, and decreased the number of primordial, primary, cystic and atretic follicles. It also helped to regulate the estrus cycle and improved the quality of the ovary.

Conclusion: Seminal plasma and hCG can increase the number of corpus luteum and induce ovulation in the polycystic ovary. Metformin is more effective in follicles growth, but does not have more effect on ovulation induction.

Keywords: Human Seminal Plasma, Polycystic Ovary, hCG Hormone, Metformin

P-69: Methanolic Extract of Coconut Meat Decrease Apoptosis of Testis by Improvement of Oxidative Stress in Type II Diabetic Rats

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Background: The increasing incidence of diabetes mellitus (DM) and its complications has made this disease one of the biggest health threats to the communities. There is a strong association between male infertility and DM and about 90% of male diabetic patients have abnormalities in the fertility. Coconut is one of the highest nutritional and medicinal value plants and our previous studies showed that methanolic extract of coconut meat (MECM) can improve quality and quantity indices of sperm in diabetic rats. This study was conducted to evaluate the effect of MECM on apoptosis and oxidative stress in testis of type II diabetic rats.

Materials and Methods: Twenty-five adult male Wistar rats were divided into 5 groups including control, Diabetic and 3 treated diabetic groups which received 100, 150 and 200 mg/kg/day MECM by oral gavage for 40 consecutive days. Type II diabetes was induced by high fat diet and 35 mg/kg streptozotocin. Finally, animals were euthanized and their left testis was removed. Tissue samples were processed by routine and standard paraffin embedding and sectioned. Detection of apoptotic cell was performed by nonradioactive in situ end labeling method using TUNEL immunocytochemical technique. A part of testis tissue were homogenized and level of glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured. Finally, data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc ($\alpha=0.05$).

Results: The results showed that induction of diabetes significantly increased Apoptotic indices and MDA level of testis but decreased GPx, SOD and TAC level of testis while in all treatment groups, these conditions were improved in a dose dependent manner. Our results indicated that administration of MECM in all three dose reduced apoptotic indices and MDA level of testis and also increased GPx, SOD and TAC level of testis tissue significantly compared to diabetic groups ($P<0.01$). There is no significant difference between mid and high dose of MECM groups and control groups in these regards ($P>0.05$).

Conclusion: Based on our results it can be concluded that administration of MECM can decrease apoptosis in diabetic patients by reduction of oxidative stress in testis tissues.

Keywords: Apoptosis, Coconut, Diabetes, Oxidative Stress, Testis

P-70: Evaluation of Sperm Parameters and Lipid Peroxidation in Male Rats Induced by Varicocele

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Background: Varicocele is introduced as one of the main causes of infertility in men and is accompanied with the abnormal dilatation, elongation and tortuosity of the pampiniform plexus veins of the spermatic cord. Several studies showed that level of oxidative stress is high in infertile men with varicocele and is considered as a key element in the pathophysiology of varicocele. Therefore, in this study, we aimed to compare sperm parameters and lipid peroxidation as oxidative stress marker in varicocele rats.

Materials and Methods: For this study, 30 adult male Wistar rats were considered and varicocele induction was performed on 10 male rats. Twenty rats were considered as control and sham groups. After two months of varicocele induction, all the rats were sacrificed and epididymides were dissected. Then, sperm parameters and sperm lipid peroxidation were assessed by WHO protocol, and Bodipy staining. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Turkey). Collected data were presented as mean \pm standard error of mean (SEM) and $P<0.05$ was considered to be significant.

Results: The result of this study showed that mean of sperm concentration, motility and morphology significantly decreased

in varicocele induction group compared to control and sham groups ($P < 0.05$), while mean percentage of sperm lipid peroxidation significantly increased in varicocele rats compared to control and sham groups ($P < 0.001$).

Conclusion: Therefore, low quality of sperm parameters in varicocele status might be associated with the role of seminal reactive oxygen species in mediating such damage.

Keywords: Varicocele, Lipid Peroxidation, Sperm

P-71: How Much Sperm Head Vacuole Affects Outcome of ICSI in Infertile Men?

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Background: The better understand from concept of “sperm competence” can promote the clinical outcomes under assisted reproductive techniques. Sperm morphology is a potential marker of sperm competence that reflects the intrinsic quality of sperm. One of the noteworthy parameters of sperm morphology is sperm head vacuole.

Materials and Methods: To assess whether the evaluation of sperm head vacuole with standard sperm morphology assessment can predict intracytoplasmic sperm injection (ICSI) outcomes, a study was conducted between October of 2017 to March of 2018. A total of 42 ICSI cycles for male infertility factors with vacuolated spermatozoa were included. The frequency (one or more than one) and size (small or large) was evaluated using scanning electron microscope (SEM) for each patient. The classification of each semen sample was performed based on Vanderzwalmen's criteria: grade I, no vacuoles; grade II, ≤ 2 small vacuoles; grade III, ≥ 1 large vacuole; grade IV, large vacuole with other abnormalities. The assisted reproduction outcomes such as rates of fertilization, embryo development, and biochemical pregnancy were followed for each cycle. CorrelationAttributeEval and Ranker of WEKA software was used to evaluate the effect of each feature/factor of grade on biochemical pregnancy rate.

Results: The examination of sperm head vacuole was not significantly associated with fertilization rate and embryo development rate ($P > 0.05$). While this association was significantly observed on biochemical pregnancy rate. The effect of grade IV was weighed more than other parameters on declined biochemical pregnancy.

Conclusion: Precise evaluation of sperm parameters with conventional assessment can help embryologists to select the best sperm and improve assisted reproductive outcomes.

Keywords: Vacuolated Spermatozoa, Chromatin Status, Morphology, Fertilization, Pregnancy

P-72: Protamination and Acrosome Integrity in Vacuolated Human Spermatozoa

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Background: More DNA damage was documented in infertile men's sperm than those of fertile men. The underprotamination, the abnormal chromatin packaging, may induce DNA damage. The aim of this study was to determine the relationship between the frequency of large nuclear vacuoles and protamine-deficient spermatozoa, and reacted acrosome rate.

Materials and Methods: This study was including 42 men with male factor infertility and 22 men with normal semen in an assisted reproductive cycle between October of 2017 to February of 2018. The frequency (one or more than one), size (small or large), and acrosome position (nuclear or non-nuclear) was evaluated using scanning electron microscope (SEM). The protamine status was evaluated with chromomycin A3 (CMA3) staining. The triple staining was used to assess the acrosome reaction. A total of 100 sperms were evaluated for the status of vacuole, protamination, and acrosome reaction.

Results: The presence of bright yellow fluorescence (CMA3-positive) was more frequently observed in spermatozoa with large nuclear vacuole (LNV) than other groups (2336/4200; 55.6% vs. 621/1500; 41.4%, respectively), reflecting a higher percentage of abnormal chromatin packaging in spermatozoa with LNV. Also, the presence of more than one small nuclear vacuole shows more abnormal chromatin packaging in comparison to large non-nuclear vacuole ($P < 0.05$). The percentage of reacted acrosomes (blue/white) was significantly higher in spermatozoa with non-nuclear vacuoles in comparison to other groups ($P < 0.05$).

Conclusion: This study provided a novel insight into how much sperm vacuoles negatively affect fertility sperm potential. Therefore, the observation of vacuolization of nuclear spermatozoa could reflect the presence of molecular anomalies and high reacted acrosome rate.

Keywords: Sperm Vacuole, Protamine Deficient, Acrosome Integrity, Male Factor Infertility

P-73: Effect of Human Seminal Plasma on *In Vitro* Growth of Mouse Follicles and Oocyte Maturation

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Background: *In vitro* growth and maturation of pre-antral ovarian follicles is as one of the important techniques in ART. This is a good way to preserve fertility in women whom reduced their ovarian function due to various reasons such as radiotherapy and chemotherapy. The current challenges for follicle culture are optimization of culture media to match the physiological needs of the cell *in vivo*. The present study was designed to determine whether different seminal plasma concentrations in the culture medium could improve the developmental growth of follicles and oocyte maturation.

Materials and Methods: Human seminal plasma was obtained after centrifugation (3000 g/min for 10 min). The culture medium was consisted of α -MEM supplemented with 10% FBS, 100 mIU/ml (rFSH), 0.05, 0.1 and 0.5% (v/v) concentration of seminal plasma (treatment I, II, III respectively) and control group, non-seminal plasma. All selected Pre-antral follicles (100-140 μ m) from different treatment group were mechanically isolated from the ovaries and cultured in different dishes under the same culture conditions (5% CO₂ in air at 37°C for 9 days). The follicles from the four experimental groups were assessed for viability with trypan blue staining, follicles growth, oocyte survival and oocyte maturation rate *in vitro*. Chi-square test was used for evaluating differences between control and experimental groups.

Results: Result indicated that follicle growth rate in different concentration of seminal plasma decreased significantly compared with control group over a period of 9 days. Percentage of oocyte maturation in ovulated oocytes from cultured follicles with 0.1 percentage concentration of seminal plasma increased significantly in comparison of control group ($P \leq 0.05$).

Conclusion: Seminal plasma is a dose-dependent manner that has a positive effect on *in vitro* maturation of oocytes.

Keywords: Human Seminal Plasma, *In Vitro* Culture, Pre-Antral Follicle, Mouse

P-74: Effects of Iron Oxide Nanoparticles on Mouse Sperm Parameters and Testicular Tissue

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Background: Iron oxide nanoparticles are commonly used in various areas such as biomedical, medicine, and cosmetics. There is a little information about the effects of the nanoparticles on human health. The current investigation was conducted to evaluate the negative effects of these compounds on several reproductive parameters in mice.

Materials and Methods: Sixteen male NMRI mice randomly divided into four groups. Control mice received only a regular diet. Three experimental groups were administered for four days with FE203 NPs, in doses of 50, 150 and 300 mg/kg intraperitoneally (i.p.). Epididymal sperm parameters such as sperm number and motility parameters were assessed by computer-assisted sperm analysis (CASA). Stereological analysis was also done on histological sections.

Results: The results demonstrated that FE203 NPs caused a significant decrease in sperm motility, sperm motion parameters (VCL, VSL, VAP), total number of the spermatogonia, primary spermatocyte, spermatid, Sertoli, Leydig cells and also the total length of seminiferous tubules, volume of testis (VT), volume of interstitial tissue (Vit) in dosage of 300 mg/kg/day.

Conclusion: In summary, iron oxide nanoparticles can affect several reproductive parameters in highest dosage. Further studies are needed to understand the mechanism of action of these nanoparticles on the reproductive system.

Keywords: Iron Nano-Particles, Sperm Parameters, Testicular Tissue, Mice

P-75: The Effect of Various Concentrations of Eggplant Peel Extract on Quality Membrane Integrity Farahani Ram Sperm Breed

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Background: This study was conducted by investigating the effect of different concentration eggplant peel extract (0, 2, 4, 6 and 8 percent/ml) in extender with based Tris-egg yolk on the experiment Farahani ram breed. Semen samples were collected from five mature Farahani ram breed twice in the week by an artificial vagina, after equilibration at five degrees centigrade the samples, sealing by 0.5 straw and were frozen with nitrogen vapor and immersed in liquid nitrogen and were stored by using for assessment. Semen's were thawed at 37°C and then assessed for analysis of membrane integrity (HOST) by computer analysis (CASA). The result of this experiment showed that the extender containing about 2 percent/ml of eggplant peel extract significantly improved cell membrane integrity compared to control group ($P < 0.01$). After that, the extenders containing about 4 percent/ml from eggplant extract peel and control groups had an equal relationship of significant ($P < 0.01$). With the attention, this conclude of this experiment addition 2 percent/ml of eggplant peel extract to Tris extender could be suitable for preservation of cell membrane integrity.

Materials and Methods: Semen samples were obtained from five Farahani ram around (2-3 years old) and the average weight of 50-60 kg. This experiment was carried out in October and December at experimental station of Arak University. Semen was collected with an artificial vagina at two times a week. By the way, for prevention of individual effects, the semen samples were polled and the ejaculations were immediately transferred to a water bath with 37°C for transportation to the laboratory. The value of every ejaculation was about 1-2 ml and concentration $2/5 \times 10^9$ spermatozoa/ml healthy and normal spermatozoa.

Results: The results of this study showed that extender restrains of 2 percent/ml eggplant peel extract could be decreased cell membrane integrity compared to control group after freeze-thawing, although the level of 4 percent/ml and control group at this experimentation had equal of statistical significance in cell membrane integrity ($P < 0/01$).

Conclusion: In this experiment, our results showed that using 2 percent/ of eggplant peel extract in the condition of the laboratory could improve membrane integrity and quality of spermatozoa after the freeze. However, based on the results acquired in the present study greater level addition did not grant more useful effect.

Keywords: Extract, Cell Membrane Integrity, Ram

P-76: The Effect of *In Vitro* Solanum Melongena Extract Additive to the Semen Extender on The Progressive Sperm Motility after Freezing in Ram

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Background: This study aimed of to investigate the effect of *in vitro* addition extract of Solanum melongena on freeze and post-thawing spermatozoa quality of ram (Farahani ram). In this study, five healthy rams from ideal reproduction body were used. Each sample of ram pooled together with other samples and then diluted at 37° with extender containing (control, 2, 4, 6 and 8 percent of Solanum melongena extract) and analyzed progressive sperm after freezing. Whereas, each sample cooled and balanced at 5° in 0.5 straw, then estimated semen parameter (progressive sperm) after thawing straw in water 37°. In conclusion, our result indicated that supplement 2 and 4 percent of eggplant peel extract to extender containing Tris-yolk with 3.786 g Tris, 2.172 g citric acid and 1 g fructose in 100 ml distilled water. The diluent was supplemented with 5.0% (v/v) glycerol 15.0% egg yolk, penicillin (100,000), significantly improved progressive sperm post thawing compared to control group (P<0.01). In this study, levels of control (0), 2 and 4 percent of eggplant peel extract had equal significantly (P<0.01). However, the supplement containing of 6 and 8 percent eggplant peel extract had a deleterious effect on progressive sperm motility spermatozoa after freezing.

Materials and Methods: This experiment was carried at experimental station of Arak University. Also Extender of this study provided by a company (Merk Germany). Collection of semen was from four Farahani ram, after collection, semen samples immediately passed on to the laboratory for analysis. Optimal progressive sperm motility was up 75 percent and under 10 percent progressive motility was not optimal for this analysis. In this experiment for each treatment used five repeats.

Results: In this assessment dilution containing about 6 and 8 percent eggplant peel extract didn't expos significantly and gradually decrease progressive sperm motility, but levels of control (0), 2 and 4 percent extender containing explant peel extract had same significantly (P<0.01).

Conclusion: In this study, we demonstrated the level of 2 and 4 percent eggplant peel extract in extender can be used cryopreserved. In conclusion, we implied that the extender containing level appropriate of eggplant peel extract has an effect beneficial to progressive sperm motility post-thawing.

Keywords: Extract, Cryopreservation, Spermatozoa, Progressive Motility

P-77: Protective Effect of Aloe Vera Gel Extract Against Adverse Effect of Doxorubicin on Mice Ovary

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Background: Doxorubicin is one of the most important anti-cancer chemotherapy drugs that are widely used to treat solid tumors and leukemia. This drug has side effects on most organs such as ovaries. Aloe Vera is one of the oldest medicinal plants that has high antioxidant properties. The present study show that Aloe vera gel protect from mouse ovary structure against Doxorubicin.

Materials and Methods: For this aim, female NMRI mice

were divided into four groups. Doxorubicin group received DOX 3 mg/kg/wt intraperitoneally on days 7, 14, 21, and 28. Doxorubicin/Aloe vera group received DOX 3 mg/kg/wt intraperitoneally on days 7, 14, 21, and 28 and Aloe vera gel extract 200 mg/kg/wt daily for 28 days. Aloe vera group received Aloe vera gel extract 200 mg/kg/wt daily for 28 days and control group received nothing.

Results: The Doxorubicin showed significantly decreases in the body weights and diameter of the ovary and injury to blood vessels and fibrosis of the ovarian cortex also significantly increased the number of atresia follicles at different stages of growth. Doxorubicin/ Aloe vera group showed significantly recovery in abovementioned parameters.

Conclusion: The results indicated that Aloe vera gel can improve the damage caused by doxorubicin to the ovary and protect the ovary from the harmful effects of doxorubicin.

Keywords: Doxorubicin, Aloe Vera, Ovary, Mice

P-78: The Effects of Methamphetamine on The Morphology and Motility Sperms in The Immature Male Rats

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Background: Methamphetamine is a central nervous system stimulant. The drug is abused by young Iranian. In this study, the effects of methamphetamine on the morphology and motility of sperm were evaluated.

Materials and Methods: 40 immature male Wistar Rats were divided into four groups: control and experimental: (1, 3, 5 mg/kg). The control group received saline and experimental groups received methamphetamine for 10 days (intraperitoneally) and they were allowed to mature. Then, the mice were dissected and their sperm samples were collected. Thus, the motility and morphology of sperm evaluated. The results were analyzed by ANOVA and Duncan's test and level (P<0.05) was considered significant.

Results: Average number and motility of sperm in experimental groups decreased significantly than the control group. Sperm morphological changes (reduced healthy sperm, Increasing dysfunction of flagellates, disruption in the head connection) in experimental groups compared to the control group was statistically significant

Conclusion: Frequent consumption of methamphetamine even in low doses decreased number and motility of sperm in the epididymis as well as changes in sperm morphology. This is likely to reduce fertility or sterility in males.

Keywords: Metamphetamine, Morphological changes, Sperm Motility, Sperm Number

P-79: Effects of Methamphetamine on CatSper Genes and Proteins Expression in Mouse Testis

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Background: Methamphetamine (METH) is an strong central

nervous system stimulant that has toxic effects on the reproductive system but the mechanism by which methamphetamine affects male fertility is unclear

Materials and Methods: 24 Male mice were Divided into four groups :control mices; received tap water, sham control mices; received normal saline, Experimental 1 mices; received METH at dose of 5 mg/kg of body weight and Experimental 2 mices that received METH at dose of 15 mg/kg of body weight. METH was administered on 16 consecutive days intraperitoneally (i.p).At the end of the treatment period, the mice were sacrificed and their left testis were separated. Sperm suspension was obtained from the left epididymis of animals. sperm smear and testis tissue were used for immunohistochemistry and real-time PCR analyses.

Results: Our data showed that mRNA Expression level of Cat-Sper 1 and 2 were decreased at both of low and high dose and Immunohistochemistry shows that CatSper1 and 2 reaction decreased in experimental groups.

Conclusion: Reduction of CatSper 1,2 gene and protein expression, through reducing the calcium entering the sperm and preventing of calcium-dependent changes, may reduce the mobility of sperm and affects male fertility.

Keywords: Methamphetamine, Catsper, Male Fertility

P-80: Dual Effect of Vitamin D on Apoptosis Frequency and Its Correlation with Reactive Oxygen Species Production in Human Granulosa Cells of Normal and Polycystic Ovaries

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Background: Despite the increasing number of growing follicles in PCOS patients, pathways and frequency of apoptosis in cystic follicles is controversial. Vitamin D directly or indirectly influence on genes involved in cell cycling, proliferation, and apoptosis. Therefore, we investigated apoptosis frequency in normal and cystic granulosa cells (GCs) in a basal state and under vitamin D treatment and its correlation with the amount of ROS production.

Materials and Methods: GCs were obtained from 20 women with PCOS and 20 healthy controls. Ovarian GCs were cultured in presence or absence of vitamin D (100 nM), for 48 hours. ROS production (RLU) was measured by chemiluminescence assay. The apoptotic cells were identified by Annexin-V/ Propidium iodide detection kit and mean Annexin fluorescent intensity (MFI) was calculated. The comparisons were undertaken using independent t-test and Pearson correlation coefficient. All statistical analysis performed using GraphPad Prism software v. 6.0.

Results: The basal ROS generation by polycystic ovarian GCs was markedly greater than normal ones ($P < 0.0001$). Vitamin D significantly reduced ROS production in both groups. However, the ROS generation by treated GCs of patients was still significantly higher than treated normal cells. The percent of necrotic, early and late apoptotic, and live cells were similar in normal and PCOS groups. The frequency of the apoptotic cells (MFI) significantly ($P < 0.03$) decreased in vitamin D treated normal GCs, whereas it increased in treated cells of PCOS patients ($P < 0.007$). There were significant direct correlations between RLU and rate of cell apoptosis (MFI) in normal women ($P = 0.03$) and PCOS patients ($P = 0.0003$). Although ROS levels

in PCOS group was higher than the controls, there was no significant difference between apoptotic rate of GCs of normal and PCOS women.

Conclusion: Dysregulation of apoptosis-related genes may result in an attenuated atresia, consequently polycystic ovaries. Vitamin D reduced ROS level in both groups, whereas this vitamin accelerated apoptosis in GCs of PCOS group and maintained cell viability in normal cells. It can be assumed that this vitamin affects gene expression of apoptotic and anti-apoptotic factors. However, future researches are required to elucidate the exact mechanism of vitamin D action.

Keywords: PCOS, Vitamin D, Apoptosis, ROS, Granulosa Cells

P-81: Glutathione in Ram Semen Cryopreservation: Flow cytometric, Biochemical and Motion Findings for Frozen-Thawed Sperm in Extender Containing Egg yolk

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Background: The aim of this study was to assess the effects of different concentrations of reduced glutathione (GSH) in egg yolk-based extender for cryopreservation of ram semen.

Materials and Methods: Motility characteristics, membrane functionality, abnormal morphology, apoptosis status, mitochondria activity, acrosome integrity and lipid peroxidation were evaluated after cryopreservation. Semen samples were collected from 5 rams, twice a week, then diluted in the extenders that contained different concentrations of GSH as follows: extender without GSH (control, G 0), extender containing 0.5 mM (G 0.5), 1 mM (G 1), 2 mM (G 2), 4 mM (G 4) and 8 mM (G 8) GSH.

Results: Supplementation of egg yolk-based with mM GSH produced higher significant total motility (51.2 ± 1.5 %), progressive motility (30.1 ± 1.3 %), membrane functionality (46.1 ± 1.3 %), mitochondria activity (52.1 ± 1.7), acrosome integrity (67.0 ± 1.7 %), viability (50.0 ± 1.4 %) and lower significant of lipid peroxidation (3.8 ± 0.09 nmol/ml) compared to control group. MDA production was higher in G 8 (4.5 ± 0.09 nmol/ml) and other parameters was lower in G 8 compared to other groups. Different concentrations of GSH did not have any effects on sperm morphology.

Conclusion: Our results revealed that supplementation of freezing extender with 4 mM GSH significantly improved the ram sperm quality after freezing-thawing process.

Keywords: Egg Yolk, Sheep, Glutathione, Sperm Freezing

P-82: Evaluation of The Effect of Cryopreservation on Sperm DNA Fragmentation

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Background: There is a necessity for semen cryopreservation in andrology labs especially for fertility preservation before chemotherapy, radiotherapy and diseases and surgeries induced testicular failure or ejaculatory dysfunction. Therefore, evaluation of the effect of temperature variations on sperm structure

especially for DNA integrity is essential to warranty its fertility potential. The aim of present study was to evaluate the effect of cryopreservation on the sperm DNA fragmentation in men undergoing infertility investigation.

Materials and Methods: Semen specimens were collected from 111 men referring to Mehr medical institute for infertility investigations. All samples were obtained by masturbation after 3 days of sexual abstinence. Conventional semen analysis was used to measure semen volume, sperm concentration, motility, and morphology. Each liquefied semen samples were divided into two equal aliquots to compare DNA fragmentation rate in fresh and cryopreserved-thawed sperm samples. Sperm DNA Fragmentation Index (DFI) were scored using sperm chromatin dispersion (SCD) assay.

Results: The mean values for fresh semen parameters were as follows: sperm concentration $38.23 \pm 19.94 \times 10^6$, motility $61.57\% \pm 13.99\%$ and normal morphology $11.92\% \pm 4.51\%$. Only one man indicated DFI=33% after cryopreserved-thawed process. There were no significant differences in DNA fragmentation index between before and after cryopreservation-thaw samples (19.28 ± 4.65 vs. 18.83 ± 4.70 respectively, $P=0.296$).

Conclusion: We concluded that cryopreservation of semen samples did not have deleterious effects on sperm DNA integrity and is convenient method for fertility preservation.

Keywords: Cryopreservation, Fertility Preservation, DNA Fragmentation

P-83: The Effects of Time Interval between Sperm Processing and Intrauterine Insemination on Pregnancy Rate

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Background: Intrauterine insemination (IUI) is a simple and inexpensive method that has been utilized for fertility treatment of infertile couples. Because the limited survival time of spermatozoa, timing in IUI is one of clinical factors that may have influence on pregnancy rate. However, there is no consensus on the effects of time interval between sperm processing and IUI on pregnancy rate. The aim of present study was to evaluate the difference of pregnancy outcome on time interval between semen processing and IUI.

Materials and Methods: Data were collected retrospectively from one hundred twenty eight patients who were referred for infertility treatment. After conventional semen analysis, samples were prepared by swim-up method and followed by incubation at 37° C. According to time interval between sperm processing and insemination, the patients were divided into four groups: <30 minutes, 30-59 minutes, 60-119 minutes and ≥ 120 minutes. All data were compared among groups.

Results: The mean age of patients was 34.49 ± 6.98 . There was no significant difference in sperm concentration, motility and morphology before and after semen processing among four groups. No significant difference was seen in pregnancy rate among four groups (28.9, 21.4, 10, 28.6% respectively, $P=0.275$). Logistic regression analysis indicated that there was no relation between time interval between semen processing and IUI and pregnancy outcome.

Conclusion: According to the results, prolonged incubation of processed samples has no negative effects on pregnancy outcome. This attitude allows laboratories for workload management.

Keywords: Intrauterine Insemination, Spermatozoa, Pregnancy Outcome

P-84: Effect of Vanadium on Sperm Parameters in Offspring from Pregnant Mice

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Background: Trace elements are used as alternative medicine for decades. Some of the trace elements such as platinum, Gallium, Copper and Vanadium used for treatment of some diseases. Vanadium is dependent on dose and has insulin mimicking and anticancer role in low dose. In this research we investigated the effect of Vanadium on vital parameter of sperm in offspring from pregnant mice.

Materials and Methods: For this aim, we divided the pregnant mice into 2 groups, control group that received nothing and Vanadium group that received Vanadium 4 mg/kg at 8, 10, 12 days of pregnancy. 60 days after birth we sacrificed the male mice and removed the caudal epididym and incubated the suspension for 10 min in incubator.

Results: Our results showed that Vanadium decreased significantly sperm count and increased the sperm abnormality and sperm motility significantly compared to the control group, but did not show any different in sperm viability with control group.

Conclusion: So on the base of our research Vanadium can introduce as a good alternative medicine for protect the sperm viability and motility against the destructive factors.

Keywords: Vanadium, Sperm, Mice, Pregnancy

P-85: Extended Incubation Affect The Histone Modification of Mouse Spermatozoa

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Background: The histone modifications are epigenetic marks that control the developmental pattern of the preimplantation embryo and are influenced by many factors, such as the sperm and oocyte quality and gamete *in vitro* manipulation. The purpose of this study was to investigated the changes in H3K4me3 (Histone 3 lysine 4 trimethylation) and H3K27me3 (Histone 3 lysine 27 trimethylation) marks after long term *in vitro* sperm incubation at 37°C.

Materials and Methods: Mouse sperm samples incubated at 37°C for 24 hours. Before and after extended incubation H3K4me3 and H3K27me3 marks were evaluated by flowcytometry. Relative expression levels of histone methyl-transferase H3K27 (Ezh2) and histone methyl-transferase H3K4 (Mll) were assessed by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: H3K4me3 and H3K27me3 marks were significantly higher after incubation at 37°C compared with fresh sample ($P<0.05$). The level of two histone methyl-transferases genes (Ezh2 and Mll) showed significant increase between two groups

($P < 0.05$).

Conclusion: The result of this study demonstrated that extended sperm incubation affect correct histone methylation patterns. Aberrations in these epigenetic marks may have detrimental consequences for early embryonic development.

Keywords: Incubation, Histone Modification, Spermatozoa

P-86: Evaluation of Sperm DNA Fragmentation and Apoptosis after Density Gradient Centrifugation for Sperm Preparation at Different Time Intervals

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Background: One of the causes of failure in ART is sperm DNA fragmentation which may be increased by incubation of spermatozoa in 37°C. The objective was the evaluation of sperm DNA fragmentation using the sperm chromatin dispersion (SCD) test in spermatozoa after gradient at different time intervals prior to use. In this prospective study, we analyzed twenty one normozoospermia specimens. Semen analysis was performed according to WHO guidelines.

Materials and Methods: The samples were incubated at 37°C after preparation by the gradient. DNA fragmentation was 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. After direct swim-up, sperm cells were incubated at 37°C and Apoptosis was evaluated at different time intervals (0, 1, 2 and 3 h).

Results: Here was an increasing trend in sperm DNA fragmentation after incubation. No significant difference in percentage of sperm cells with fragmented DNA was seen after 1h compared to 0h (6.14 ± 0.89 vs. 4.38 ± 0.8), also 2h compared to 1h ($P=0.15$) and 3h compared to 2h ($P=0.4$). However, there was significant increase in sperm DNA fragmentation after 2h (8.81 ± 0.93 , $P=0.004$) and 3h (10.76 ± 0.89 , $P < 0.0001$), also 3h compared to 1h ($P=0.002$). The rate of Apoptosis was significantly higher after 2h compared to 0h ($9.19 \pm 0.8\%$ vs. $4.9 \pm 0.9\%$, respectively, $P=0.008$).

Conclusion: It seems that incubation of prepared normozoospermia samples at 37°C prior to use in ART should be less than 2h.

Keywords: Normozoospermia, SCD Test, Sperm DNA Fragmentation

P-87: Assessment of Sperm PAWP and DNA Damage in Infertile Men

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Background: Post-acrosomal WW-domain binding protein (PAWP) has been reported to elicit oocyte activation when injected into the oocytes, and to produce a Ca²⁺ increase in oocytes. PAWP is one of the possible perm-borne oocyte-activating factors (SOAFs) candidates and is widely conserved among eutherian mammals. The molecular mechanisms underlying the precise function of PAWP are currently unknown. Herein, the present study aimed to simultaneously evaluate the association between expression of PAWP and DNA fragmentation in fertile and in sperm of infertile men.

Materials and Methods: Seminal ejaculates from 61 healthy men volunteers (controls) and 67 infertile men (with male factor infertility) referred to an andrology laboratory. Then the sperm parameters were assessed by the WHO guidelines (2010). Samples were examined for DNA damage by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick-end labeling (TUNEL) and the expression of sperm PAWP was assessed by Real Time PCR.

Results: The quality of sperm parameters were significantly lower in the infertile men compared with the fertile men. In addition, the expression of sperm PAWP was significantly lower, and percentage of sperm DNA damage were significantly higher in infertile men than fertile men.

Conclusion: Our results indicated that low or absence expression of PAWP and high DNA damage sperm could be considered as factors involved in failed fertilization in infertile men.

Keywords: PAWP, DNA Damage, Sperm Parameters

P-88: Short Term Culture of Vitrified Human Ovarian Cortical Tissue to Assess The Cryopreservation Outcome: Molecular and Morphological Analysis

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Background: The aim of the present study was to evaluate the effectiveness of human ovarian vitrification protocol followed with *in vitro* culture at the morphological and molecular levels.

Materials and Methods: Ovarian tissues were obtained from 10 normal transsexual women and cut into small pieces and were divided into non-vitrified and vitrified groups and some of the tissues fragments in both groups were randomly cultured for two weeks. The morphological study using hematoxylin and eosin and Masson's trichrome staining was done. The analysis of mean follicular density, 17-β estradiol (E2) and anti mullerian hormone (AMH) and real-time RT-PCR was done for the evaluation of expression of genes related to folliculogenesis. Data were compared by paired-samples and independent-samples t test. Values of $P < 0.05$ were considered statistically significant.

Results: The proportion of normal follicles did not show significant difference between vitrified and non-vitrified groups before and after culture but these rates and the mean follicle density significantly decreased in both cultured tissues ($P < 0.05$). The expression of genes was similar in vitrified and non-vitrified groups but in cultured tissues the expression of GDF9 and FSHR genes increased and the expression of FIGLA and KIT-L genes decreased ($P < 0.05$). An increase in E2 and AMH concen-

tration was observed after 14 days of culture in both groups.

Conclusion: The present study indicated that the follicular development and gene expression in vitrified ovarian tissue was not altered before and after *in vitro* culture, thus this method could be useful for fertility preservation; however, additional studies are needed to improve the culture condition.

Keywords: 17 Beta- Estradiol, Anti-Mullerian Hormone, Gene Expression, Vitrification

P-89: Effect of Antioxidant Taurine on Vitrification of Human Sperm

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Background: The use of the vitrification procedure with antioxidants similar taurine for the treatment of male infertility is the stuff of the world. With all the benefits of cryopreservation, Concerns about the effects that happen during cryopreservation causing sperm cells damage structure and function. The aim of this study was to evaluate the effect of antioxidant taurine on sperm in normospermic patients during vitrification.

Materials and Methods: Men who were enrolled at Royan institute participated in this study 20 normospermic sample were assessed according to WHO 2010. After semen preparation with density gradient centrifugation (DGC), each sample was divided into two groups consist of vitrification (control) and taurine group. Total motility and progressive motility (analyzed by Computer-Assisted Sperm Analysis), morphology (stained by papanicolaou), acrosome reaction (FITC-PSA labeling) and malondialdehyde levels were studied in two groups.

Results: The results show Total and progressive motility taurine in the experimental group than the control group increased, but this change was not significant. Group taurine was not significantly better performance than the control group maintained normal morphology. Increased lipid peroxidation level in the control group compared to taurine during vitrification, it did not change significantly. Acrosome reaction was higher in control group than the taurine group but this change not significant.

Conclusion: Vitrification decrease of motility, normal morphology, acrosome intact and sperm cell increased lipid peroxidation. Therefore, the use of antioxidants such as taurine in vitrification conditions is important.

Keywords: Taurine, Vitrification, Antioxidant

P-90: In Vitro Maturation and Fertilization in Mice NMRI: Comparison of The Effect of Bovine Serum Albumin, Fetal Bovine Serum, Autologous and Allogeneic Mice Serums

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Background: Oocyte maturation is a physiological event that is essential for the successful fertilization and development of the embryo. Sera are a complex combination of factors such as growth factors, proteins, vitamins, small elements, hormones, etc. which are essential for the growth and maintenance of cells and can affect oocyte maturation and fertilization. The aim of this study was to evaluate the effect of different sera on *in vitro* maturation and fertilization in NMRI mice.

Materials and Methods: Maturation of oocytes was investigated in Alpha MEM medium with 20, 15, 10, 5, 0 allogenic and autologous mice serums, fetal bovine serum (FBS) and bovine serum albumin (BSA) after 14 to 18 hours incubation. Then mature oocytes were fertilized in different sera with a capacity sperm in MHRM medium with percentages of 20, 15, 10, 5, 0 in different sera.

Results: The results showed that the level of *in vitro* maturation increased significantly by adding serum to the culture medium (P<0.001). Also, the rate of *in vitro* fertilization increased significantly by adding serum to the culture medium (P<0.001). Among all groups, FBS 10% (80%), BSA 5% (75%), Allogeneic mice serum 20% (54%), and autologous mice serum 20% (40%); were found to have the highest maturation; BSA 5% (77%), FBS 20% (75%), in Allogeneic mice serum 20% (70%) and in autologous mouse serum 15% (64%), the highest fertilization rate was observed.

Conclusion: There are limited studies on the effects of different types of serum added to the culture medium on different stages of maturation, fertilization, and subsequent development of the oocyte. However, using inhuman serum can be a source of contaminants and infections. The results of this study showed that although BSA and FBS had better results than allogeneic and autologous sera, but it was better to use my own serum due to the possibility of transmission of infections and diseases from animals to humans and the need to reduce the costs of infertility treatment, it is better to use one's own body or the same species.

Keywords: *In Vitro* Maturation and Fertilization, Fetal Bovine Serum, Bovine Serum Albumin, Autologous Mice Serum, Allogeneic Mice Serum

P-91: The Effect of Ovarian Encapsulation on Morphology and Follicular Count of Vitrified Mouse Ovary

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Background: Ovarian cryopreservation before chemotherapy can restore fertility to women with cancer and premature ovarian failure. Maintaining of follicular reserve is mandatory after cryopreserved ovarian tissue. The purpose of this study was to determine the influence of alginate hydrogel as an ovarian scaffold on morphology and follicular count of vitrified mouse ovary.

Materials and Methods: This experimental study was carried

out on 8 weeks old female mice (NMRI). After removing the ovaries in the diestrus phase, they were divided in three groups: non vitrified ovaries (A), vitrified ovaries (B) and vitrified ovaries that has been encapsulated in alginate hydrogel with a concentration of 0.75% (C). Ovaries were vitrified according to the kagawa method and placed on cryotop before plunging in liquid nitrogen. After 20 minutes, warming was done. Follicular preservation was assessed histologically using hematoxylin and eosin staining and number of follicles compared in three groups. Data were compared by one-way ANOVA. Values of $P < 0.05$ were considered statistically significant.

Results: The total number of follicles in all groups was not significantly different. The number of atretic follicles in non-vitrified group was significantly lower than vitrified and encapsulated vitrified groups ($P < 0.05$) but there were no significant differences between B and C groups. Most of the atretic follicles were found in secondary follicles in all groups.

Conclusion: The ovarian encapsulation in alginate hydrogel with a concentration of 0.75% before vitrification, could not improve follicular preservation. However, further studies with different concentrations of alginate hydrogel are needed to determine the effectiveness of alginate hydrogel to reduce the adverse effects of vitrification.

Keywords: Vitrification, Alginate Hydrogel, Ovary, Mouse

P-92: Investigation The Fertility Power by Intrauterine Insemination after Testicular Torsion/Detorsion in Adult Rat

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Background: This experimental study used a rat model to investigate the quality of sperm and fertility power by intrauterine insemination after Testicular torsion/detorsion in the adult rat.

Materials and Methods: In our experimental study, were used 14 male and 14 female Wistar rat. That the Male rats randomly divided into 2 groups; G1, Sham group; G2, testicular torsion for 4 hours followed by detorsion 24 h(TD). Their blood sampling, blood levels of testosterone, some oxidative stress markers and anti-oxidant enzymes were assayed. The sperm parameters including concentration, vitality, motility, and morphology were assayed. Also, the power of fertilization was investigated by intrauterine insemination in the adult female rat.

Results: The histological parameters showed a significant change in the G2 group as compared with Sham group. The levels of Testosterone, GPX, and superoxide dismutase significantly decreased in G2 group, and the malondialdehyde level increased in the duration of ischemia increased. The sperm quality and fertility power were significantly decreased in G2 when compared with Sham group.

Conclusion: The results of the present research show that the testicular torsion/detorsion have a negative effect on testis tissue, sperm quality and reduce the power of fertilization.

Keywords: Torsion/Detorsion, Testis, Fertility, IUI

P-93: Protective Effects of Ceratonia Silique Extract in Mice Reproductive System following Toxicity of Lead

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Background: Lead as a factor in reducing reproductive function and changes in hormones apoptosis are known. Potential mechanisms underlying Lead-induced reproductive and developmental toxicities, including chromosome and DNA damage (genotoxicity), oxidative stress, altered level and/or function of enzymes, hormones and proteins, apoptosis, were identified. Ceratonia silique is used medicinally and as a culinary spice. Ceratonia silique preparations have been described to exhibit total and LDL cholesterol lowering properties in hypercholesterolemic patients, antioxidant properties and an elicitation of natural antioxidant defenses, as well as beneficial effects in energy intake and body weight. The present study was conducted to assess whether Ceratonia silique extract with antioxidant properties could serve as protective agents against reproductive system following toxicity of Lead in a mice model.

Materials and Methods: Forty-six adult male mice were randomly divided into control group (n=8); sham group (10 mg/kg normal saline) (n=8); Lead group (1000 ppm) (n=8); Group 4: Ceratonia silique group (500 mg/Kg/day, oral) + 10 mg/kg FA (n=8); Group 5 Ceratonia silique group (1000 mg/Kg/day, oral) (n=8); Group 6: Ceratonia silique group (500 mg/Kg/day, oral) + Lead (1000 ppm) (n=8) and Group 7: Ceratonia silique group (1000 mg/Kg/day, oral) + Lead (1000 ppm) (n=8). Animals were kept in standard condition. Mice on experimental and control groups were killed under anesthetic conditions after 35 days. Sperm count, motility, morphology, viability and DNA integrity (AO), were studied, and total antioxidant capacity (TAC), Glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT), Malondialdehyde (MDA) levels and testosterone, LH, and FSH were measured.

Results: The Ceratonia silique groups showed significant increased ($P < 0.05$) in semen parameters. Furthermore, a significant increased ($P < 0.05$) in testosterone, LH, FSH and total antioxidant capacity were observed in the Ceratonia silique mice. In addition, the TAC, GSH, SOD, GPx and CAT levels significant increased ($P < 0.05$) in the Ceratonia silique mice. Also, MDA levels significant reduced ($P < 0.05$) in the Ceratonia silique mice.

Conclusion: Notably, administration of Ceratonia silique extract with the concentration of 1000 mg/kg b.wt is able to protect the male reproductive system of mice against lead induced toxicity.

Keywords: Ceratonia Silique, Lead, Reproductive System, Mice

P-94: The Antioxidant Effect of Royal Jelly of Oxidative Damage and Expression of Apoptosis Gene in Male Mice following Toxicity of Lead

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Background: Lead as a factor in reducing reproductive function and changes in hormones apoptosis are known. Royal jelly is stated to have, anti-inflammatory and antioxidant. The present study was conducted to assess whether royal jelly could

serve as protective agents against testicular toxicity induced by lead in mice.

Materials and Methods: Fifty-four adult male mice were randomly divided into control (n=8); sham (10 mg/kg normal saline); lead (1000 ppm); 4: royal jelly (100) + lead; 5: royal jelly (250) + lead; 6: royal jelly (500) + lead; 7: royal jelly (100); 8: royal jelly (250); 9: royal jelly (500). Animals were killed after 35 days and Sperm parameters total antioxidant capacity levels and testosterone, LH and FSH levels and expression of bak gene were measured.

Results: The royal jelly groups showed significant increased (P<0.05) in semen parameters. Furthermore, a significant increased (P<0.05) in testosterone, LH, FSH and total antioxidant capacity were observed in the royal jelly mice. In addition, expression of the apoptotic gene was significantly (P<0.05) lower in all treated (sham, royal jelly groups) and control groups compared to lead group.

Conclusion: The results showed that royal jelly extracts coadministration caused a considerable recovery in sperm parameters. Also, royal jelly extracts could protect the sperm by decreasing the expression of apoptosis genes.

Keywords: Royal Jelly, Sperm Quality, Lead, Mice

P-95: Nano Curcumin as A Promising Agent for Diminution of Oxymetholone induced Reprotoxicity in Female Mice

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Background: Oxymetholone (OXM) is an active nutritional 17 α -alkylated anabolic -androgenic steroid, which widely used by athletes as performance enhancer in high doses. On the other hand, Curcumin is a hydrophobic compound that naturally is found in the rhizome of Curcuma Longa. The Curcumin antiviral, antibacterial, antifungal, anticancer, anti-inflammatory and anti-oxidant properties has confirmed by several studies. Additionally, the vitamin E as a fat-soluble component, is a known antioxidant against reactive oxygen species in biological systems. Therefore, the current study was performed in order to reveal the ameliorative effects of Nano-curcumin and vitamin E as the antioxidant components against OXM-induced damages on *in vitro* fertilization (IVF) outcome.

Materials and Methods: In order to perform current study, thirty adult female mice were randomly categorized into experimental and control groups. The experimental group subdivided into five groups, which orally received vitamin E-sole (100 mg/kg.bw), NCMN-sole (15 mg/kg.bw), Oxymetholone-sole (5 mg/kg.bw), vitamin E (100 mg/kg.bw) +oxymetholone (5 mg/kg.bw), NCMN (15 mg/kg.bw) + Oxymetholone (5 mg/kg.bw). After 21 days, the oocytes were picked-up by inducing super ovulation (10 IU PMSG and 10 IU hCG) and underwent *in vitro* fertilization process using fresh sperms from intact mice. Then, twenty-four hours after insemination, oocytes were monitored by an inverted microscope and formation of the pronuclei and polar bodies was recorded to evaluate fertilization rate. Blastocyst development rate was evaluated by determining the number of embryos that had reached the blastocyst stage after 72 hours' incubation.

Results: Results showed significantly reduction in IVF out-

comes in OXM received groups versus other groups(P<0.05). MII oocyte number was decreased in OXM group and its improvement was not observed in treated groups. Cleavage rate on day 2 was significantly improved in Vit-E treated group only (P<0.05) and cleavage rate at blastocyst stage was not significantly improved in the treated groups.

Conclusion: The present study reveals that the OXM impacts negatively on IVF outcomes of mice oocytes and demonstrated that Vitamin E and Nanocurcumin could not overcome these effects. Although our treatments showed no improvement in IVF outcomes of OXM-induced mice, but Vit-E had better results which can use in strategies against destructive effects of OXM.

Keywords: Oxymetholone, Vitamin E, Nano Curcumin, IVF, Embryo

P-96: Effects of Myoinositol in The Cryopreservation Process of Human Spermatozoa Parameters, Oxidative Stress and DNA Fragmentation

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Background: Cryopreservation has been extensively used in assisted reproductive technology. Due to the widespread development of assisted reproductive technologies, an important task is to improve current methods of sperm cryopreservation. Inositol is a component of the vitamin B complex. Myoinositol is the most biologically important form in nature. It is involved in several systemic processes and in mechanisms of signal transduction in the plasma membrane as precursor of second messengers. On the male reproductive function, MYO appears to regulate seminal plasma osmolarity and also sperm maturation, motility, capacitation and acrosome reaction. Recently an antioxidant action has also been suggested. The aim of this study is to evaluate the beneficial effect of MYO supplement in freezing media on the post thaw sperm quality.

Materials and Methods: Semen samples from 40 normozoospermic men were divided into two aliquots and frozen with 2 mg/ml MYO free /or supplemented freezing medium. Post thaw process, computer-aided semen analysis was used to analyze sperm motility and morphology. Reactive oxygen species was evaluated by the fluorometry of DCFH-DA, as well as total antioxidant capacity were measured based on colorimetric assay by ELISA reader. Eventually, DNA fragmentation was assessed using TUNEL staining.

Results: This study revealed that, MYO significantly improved progressive motility and normal morphology in treated samples (P<0.05). While MYO did not affect the amount of ROS (P>0.05), it was associated with high values of total antioxidant capacity (P<0.05). DNA integrity was significantly affected by MYO, as in MYO treated samples, DNA fragmentation was decreased compared to control ones (P<0.001).

Conclusion: The findings support the use of 2mg/ml myoinositol supplemented freezing media in sperm cryopreservation to increase sperm quality after freezing-thawing procedures.

Keywords: Myoinositol, Human Sperm Cryopreservation, Sperm Parameters, Oxidative Stress, DNA Fragmentation

P-97: Ovarian Tissue Cryopreservation in A Young Girl with Acute Lymphocytic Leukemia before Hematopoietic Stem Cell Transplantation: A Case Report

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Background: Nowadays, there is a strong interest in the long-term effects of chemo or radiotherapy on the future fertility of the cancer patients. One common side effect of curative chemo/radiotherapy is ovarian toxicity. Prior to chemotherapy, the patients should be referred for fertility preservation. But, many patients do not receive consultation regarding the potential adverse effects of treatment on fertility and fewer are referred for fertility preservation program. There are some options for female fertility preservation prior to cancer therapy such as emergency IVF and embryo freezing that is the most successful choice and others are oocyte and ovarian tissue cryopreservation that are still at the experimental level. Evidence-based clinical practice guidelines (CPGs) are needed to reach high quality uniform care in the cancer patients and to decrease divisions in practice and costs. Many guidelines describe the circumstances under which fertility preservation is needed to be discussed and for which patients the ovarian cryopreservation may be appropriate.

Materials and Methods: A 22-year-old girl diagnosed with Acute Lymphocytic Leukemia (ALL) was referred to our institute for ovarian cryopreservation. The patient had undergone 15 sessions of chemotherapy with 30 mg Vincristine and 975 mg Adriamycin before referring for fertility preservation. The next plan for her was hematopoietic stem cell transplantation (HSCT) Because she was single, actually the best approach for the patient as a young adult was cryopreservation of the gametes. We planned for vitrification of oocytes and ovarian tissue.

Results: An AMH was also requested which was within the normal range (3.66ng/ml). It showed pre-menopausal ovarian function. follicular density was 7.48 in the fresh segment.

Conclusion: Regarding referring the patients to the fertility preservation program, a cumulative of factors such as age, diagnosis of disease, and treatment protocol should be considered, as well as a history of gonadotoxic treatment.

Keywords: Fertility Preservation, *In Vitro* Maturation, Ovarian Cryopreservation

P-98: Determining The Effective Dose of GDF9β on The Folliculogenesis and Angiogenesis, in The Grafted Ovarian Tissues onto The Chick Embryo Chorioallantoic Membrane

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Background: Ovarian tissue cryopreservation is an important field in the women fertility preservation. But one of the im-

portant challenges is disruption of folliculogenesis after transplantation. So many studies tried to identify factors that can improve the follicular development and growth after transplantation. One of the methods of ovarian tissue culture is culture on chick chorioallantoic membrane (CAM), as a borderline system between *in vitro* and *in vivo*. It is useful for assessment of different agents' effects on the transplanted ovary and follicular development. The aim of this study was to investigate the effect of GDF9β supplementation on the sheep ovarian tissues, grafted onto the CAM. We wanted to determine the effective dosage and survey the progression of folliculogenesis and angiogenesis in the grafted tissue.

Materials and Methods: Whole Sheep ovaries were collected from a local abattoir and transported to the laboratory. Ovarian tissues were prepared. Cortex was dissected from the medulla and cut into approximately 4 × 4 × 2 mm sections. Fertilized eggs of Ross chickens were purchased from a local hatchery. After transport to the laboratory, they were incubated at 37°C with 60% humidity. On 6th day of incubation, a hole was made on the blunt-pole of the eggs with a drilling machine. Using small curved scissor, a 1.5-2 cm window was created in the shell. On 7th day of incubation, prepared ovarian pices (Ops) were placed onto the large blood vessels of the CAM. 10 μl of ovarian culture medium was added to ops thrice a day. On the basis of adding GDF9β, we had two experimental groups (+ and - GDF9-β). The added dosages were 150, 200 and 250ng/ml. After 5 days of culturing, Ops were retrieved. Histological studies and immunohistological surveys with Ki-67 antibody were done.

Results: Although in both groups of culture (-GDF9β & +GDF9β) the rate of successful transplantation was the same, the number of vessels were higher in the +GDF9β groups. Also, in these groups, better follicular structure and stromal integrity was preserved. Furthermore, the percentage of the primordial follicles was decreased, whereas the number of intermediate and primary follicles was increased which is due to the progress of folliculogenesis. Although all the mentioned parameters were higher in the +GDF9β groups, the result was significant in the two dosages of 200 and 250 ng/ml. On the bases of Ki67 immunohistological analysis, the proliferative activity was preserved in both groups.

Conclusion: We concluded that folliculogenesis took place when ≥200 ng/ml of GDF9β was added to the grafted tissues. As it mentioned that suppressing effect of anti-müllerian hormone (AMH), secreted by chick gonads, may be one of the factor that inhibits folliculogenesis, the positive effect of GDF9β may be due to the suppression of AMH. So, GDF9β, follow the higher number of vessel formation and reducing the hypoxia during the transplantation, may lead to the better transplantation and follicular development.

Keywords: GDF9β, Ovarian Culture, Chick Embryo Chorioallantoic Membrane

P-99: The Effect of Hypothyroidism on The Sperm Count and Motility in Rats

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Background: The effect of thyroid hormones on the metabolism and growth regulation has long been proved. The presence of the thyroid hormone receptors in the rat and human testes has been demonstrated. Many studies on the prepubertal testicular development and subsequent spermatogenesis have also been performed, but there are few studies on the effects of post pubertal hypothyroidism on the sperm count and motility and some controversies are exist as well. The aim of this study was to investigate the effect of propylthiouracil induced hypothyroidism on the mobility and sperm count in rats.

Materials and Methods: For this purpose, male Wistar rats were randomly divided into two groups. The control (n=6) and hypothyroid (n=6) animals had free access to standard food and water and kept in standard conditions of 12-hour light/dark cycle. Hypothyroidism was induced by the use of propylthiouracil (0.025%) in the drinking water. After 5 weeks, the animals were anesthetized by ether, the left tail epididymis removed and placed in 5 ml Ham's F-10 Culture medium, and chopped. The medium kept in a 37°C incubator. After 15 minutes the sperm motility was observed and then sperm numbers per milliliter was determined using Neubauer Chamber.

Results: The results showed that the percentage of the rapid progressive sperms in the hypothyroid group (9.9 ± 3.8) (Mean \pm SEM) was significantly ($P < 0.01$) lower than the percentage in the control group (32.7 ± 5.5). The percentage of non-motile sperms in the hypothyroid animals (50.9 ± 6) was significantly ($P < 0.01$) higher compared to the control group (24.4 ± 2.3). The total sperm numbers from left epididymis in the hypothyroid group was significantly lower in comparison to the control group.

Conclusion: In conclusion, propylthiouracil induced hypothyroidism reduces sperm motility and sperm production in the rats.

Keywords: Hypothyroidism, Sperm Motility, Sperm Count

P-100: The Study of The Quality of Sperm DNA in Semen Samples Processed in Three Ways: Passing Through The Cumulus Column, Concentration Centrifugation and Incubation with Supernatant of Mesenchymal Stem Cells Derived from Fat Tissue.

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Background: Recently, the measurement of the common parameters of semen for evaluation of sperm quality is less important because these consideration cannot guarantee the absence of male infertility factors. Nowadays Clinical evidence suggests that DNA damage to sperm DNA be considered a disadvantage for reproductive results. Therefore, further studies are needed to determine a semen preparation method with minimal damage to the DNA of the sperm nucleus and its effect on reproduction.

Materials and Methods: In the present study, of infertile men semen samples with asthenoteratozoospermia who referring to infertility treatment center were processed in three ways: DGC, cumulus column, and SPAS. The results of sperm parameters and sperm count DNA fragmentation before and after the process were statistically analyzed.

Results: The results showed that the number of natural mor-

phology sperms isolated during the SPAS and the cumulus pill was significantly more than the corresponding population in the DGC group. In addition, although all three methods have the same ability to increase total sperm movement and the number of recovered sperm, in the field of forward movement and DNA fragmentation, the SPAS method functioned more efficiently ($P \leq 0.05$).

Conclusion: Considering that it has been shown that the sperm capacity is increased during the SPAS method. On However, the rearrangement of sperm chromatin by reducing the disulfide bridges and providing the possibility of re-histone over capacity, causes a significant reduction in DNA fragmentation which is not expected in this way.

Keywords: SPAS, DGC, Cumulus Pill, DNA Fragmentation

P-101: Combined Effects of Melatonin and L-Carnitine on Mouse Oocyte Morphology, Maturation and Apoptosis in Culture Condition

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Background: Melatonin and L-carnitine are the free radical scavenger, anti-apoptotic and anti-oxidant agents that are contributed to the improvement of oocyte development. The aim of this study was to evaluate the role of combination application of the two anti-oxidant agents on oocyte morphology, maturation, and apoptosis in mice.

Materials and Methods: Oocytes were harvested from mice ovaries after overstimulation, and after treatment of the cells with a combination of melatonin and L-carnitine, they were assessed for morphology, apoptosis, and maturation.

Results: Combination therapy increased oocyte diameter ($P \leq 0.003$ vs. control). The highest percentage of oocyte cytoplasmic pattern belonged to the L-carnitine group. Melatonin treatment showed the lowest rate of DNA fragmentation and the highest rate for the number of oocytes and their maturation rate.

Conclusion: Although, we found almost no significant differences between combination therapy with using either melatonin or L-carnitine alone, our results are in favor of melatonin administration as a better choice for the promotion of oocyte maturation *in vitro*.

Keywords: *In Vitro* Maturation (IVM), Oocyte, L-Carnitine, Melatonin, Mouse

P-102: Antifreeze Protein III, Ice Blocker, and Its Effect on Sperm Freezing/Thawing

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Background: Cryopreservation is a process of preserving biological function by freezing and storing material below -80°C , typically at or near the temperature of liquid nitrogen. Cryopreservation sperm, is one of the most critical procedures to preserve the reproductive capacity of individuals but the challenge in formulation successful cryoprotective agents is to design sperm freezing solutions that are non-toxic but allow for freezing at realistic cooling and warming. Naturally occurring antifreeze protein / glycoproteins which mitigate heterogeneous nucleation by binding to nucleators are reported in protection of some organisms. AFPs act by depressing the freezing point, modifying the ice-crystal formation process, preventing recrystallization, and interacting with plasma membrane at low temperatures, thus allowing these species to survive in waters colder than the equilibrium freezing point of their internal fluids. We hypothesized that addition of antifreeze protein III (AFPIII) to the extender will improve the viability, sperm progressive motility (SPM), plasma membrane integrity (PMI) and DNA fragment index (DFI) of cryopreserved human sperm.

Materials and Methods: Ejaculated semen was collected from 20 normospermic male and divided into four AFPIII supplemented groups (0.01, 0.1, 1, 5, $10\mu\text{g}/\text{mL}$) and one control group without AFPIII and supplementation. Semen samples were treated with Glycerol-egg-yolk-citrate medium (1:2, at 37°C , final concentration: 20×10^6 sperm/mL) contained mentioned concentrations of AFPIII, packed into straws. The straws were exposed to liquid nitrogen (LN2) vapor for 10 min and then plunged into the LN2 after a week, straws were thawed at 37°C for 30s and then viability, SPM, PMI, DFI and measurement of ATP were assessed.

Results: The percentage of sperm viability, SPM, PMI and ATP was higher ($P>0.05$) and DFI was lower in $1\mu\text{g}/\text{mL}$ AFP III group compared to control group. Sperm viability decreased ($P>0.05$) in 0.01, 0.1, 1, 5, $10\mu\text{g}/\text{mL}$ AFPIII groups. Other AFPIII concentrations did not show any positive effects on SPM, PMI, DFI and ATP.

Conclusion: supplementation of the extender with $1\mu\text{g}/\text{mL}$ AFPIII can improve the results and efficiency of human sperm cryopreservation.

Keywords: Cryopreservation, Antifreeze Protein III, Spermatozoa

P-103: Antifreeze Protein III, An Ice Blocker, and Its Effect on Sperm Freezing/Thawing

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Background: Sperm cryopreservation is one of the most critical strategies to preserve men reproductive potency. Designing an appropriate sperm freezing medium that be non-toxic and allow successful freezing is a big challenge. Antifreeze proteins (AFPs) have been used in different scientific researches such as; cryopreservation of cells, tissues and organs. AFPs depress the freezing point, modify the ice crystal formation process, prevent recrystallization and interact with plasma membrane at low temperatures. Our aim was to evaluate the effect of AFP III on post thaw plasma membrane integrity (PMI), Reactive Oxygen Species (ROS) and Total Antioxidant Capacity (TAC) of cryopreserved human sperm.

Materials and Methods: Ejaculated semen was collected from 20 normospermic male and each sample was then divided into three groups: 1. fresh control that analyzed immediately after liquefaction time, 2. cryopreservation without AFP III that cryopreserved with Glycerol-egg-yolk-citrate (GEYC) medium and, 3. cryopreservation with AFPIII that cryopreserved with GEYC medium contained $1\mu\text{g}/\text{mL}$ of synthetic peptid AFPIII. Samples were packed in to straws exposed to liquid nitrogen (LN2) vapor for 10 min and then plunged into the LN2. After a week, straws were thawed at 37°C for 30s and then PMI, ROS and TAC were assessed.

Results: The results showed that the level of ROS was increased dramatically following freezing and thawing. However, the level of ROS in cryopreservation AFPIII+ group was significantly less than cryopreservation AFPIII - group. A significant decrease in TAC was observed in both cryopreservation groups. TAC was higher in cryopreservation AFPIII+ group than AFP III - group. Also, with the supplementation of AFPIII, significant improvement in PMI was recorded compared to AFPIII - group and decrease in PMI was seen compared to fresh control.

Conclusion: The effects of cryoinjuries were determined on mitochondrial function, motility, morphology and viability of ejaculated human sperm. This study emphasized on the beneficial effects of $1\mu\text{g}/\text{mL}$ AFPIII in the extender on post - thaw quality of human sperm.

Keywords: Cryopreservation, Antifreeze Protein III, Spermatozoa

P-104: The Relationship between Maternal Nutrition and Offspring's Sexual Performance: Stereological Technique

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Background: Maternal nutrition may have a crucial impact on the development of the fetus during pregnancy. The current study was designed to determine these effects.

Materials and Methods: Sixty mature female mouse were divided in to ten groups (n=6): I. Control (CTR; standard diet prenatal (PRE) and postnatal (POST) period); II. Prenatal-fish oil (PRE - FO) gavages $0.01\text{ ml}/\text{d}$ mother FO during prenatal; III) postnatal -fish oil (POST- FO) gavages $0.01\text{ ml}/\text{d}$ mother FO during postnatal; IV(Prenatal-Postnatal-fish oil (PRE-POST-

FO) gavages 0.01 ml/d/ mother FO during prenatal and postnatal; V) Prenatal-fish oil-vitamin E (PRE - FO+ VITE) gavages 0.01 ml/d/ mother FO + VITE (2-fold) during prenatal; VI. (POST- FO+ VITE) gavages 0.01 ml/d/ mother FO + VITE (2-fold) during postnatal; VII. PRE-POST-FO+ VITE gavages 0.01 ml/d/ mother FO + VITE (2-fold) during Pre and Postnatal; VIII. PRE-VITE; consumed VITE (2-fold) during prenatal period; IX. POST-VITE ; consumed VITE (2-fold) during postnatal period; X. PRE-POST-VITE; consumed VITE (2-fold) during pre and postnatal. After puberty, testis processed and the changes of testicular tissue were estimated using stereological methods.

Results: Spermatogonia, spermatocytes, spermatid, sperm, sertoli and leydig cells in POST-VITE and POST- FO+VITE groups significantly increased than other groups ($P<0.05$). On the other hand the number of spermatogonia, sertoli and leydig cells in PRE - FO group significantly decreased in compared with other groups ($P<0.05$). Progressive movement in the PRE-POST-FO+VITE group was significantly higher than the PRE-POST -VITE and PRE- VITE ($P<0.05$). The number of Leydig cells were lower ($P<0.05$) in CTR group than PRE-VITE, POST-VITE, POST- FO+ VITE and PRE-POST-FO+ VITE. The number of Leydig and sertoli cells were higher in POST-VITE and POST- FO+ VITE groups than PRE - FO+ VITE and PRE-POST-FO+VITE ($P<0.05$)

Conclusion: For the first time, we observed the positive effects of supplementation maternal diet by FO with VITE or sole VITE on sex sells of male offspring after puberty. The maternal omega-3 consumption without vitamin E had destructive effects on testes.

Keywords: Fish Oil, Maternal Nutrition , Testis, Vitamin E

P-105: Viability and DNA Integrity Improvement by Adding [6] -Gingerol to Freezing Medium

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Background: Cryopreservation of sperm is a valuable method for maintaining reproductive performance in men with cancer or people with sperm abnormalities such as azoospermia and oligospermia. Despite the successes achieved in this method, during this process, sperm injuries happen that reduce the percentage of sperm viability. Our aim was to investigate the effects of [6] -Gingerol as an antioxidant during the sperm freezing and thawing process. Samples were collected from 20 normospermic men referred to the Infertility Institute and Research Center and classified into two groups: freezing (control) group and freezing with a medium supplemented with 10 μ M of [6] -Gingerol.

Materials and Methods: Cryopreservation of sperm is a valuable method for maintaining reproductive performance in men with cancer or people with sperm abnormalities such as azoospermia and oligospermia. Despite the successes achieved in this method, during this process, sperm injuries happen that reduce the percentage of sperm viability. Our aim was to inves-

tigate the effects of [6] -Gingerol as an antioxidant during the sperm freezing and thawing process. Samples were collected from 20 normospermic men referred to the Infertility Institute & Research Center and classified into two groups: freezing (control) group and freezing with a medium supplemented with 10 μ M of [6] -Gingerol.

Results: Our results revealed that freezing with [6] -Gingerol increase sperm viability in comparison with control group (64.05 ± 1.32 and 56.45 ± 1.26). Whereas this antioxidant had little effect on motility in freezing with [6] -Gingerol group. Finally DNA integrity was higher in treated group (62.35 ± 1.27) as compared to control group (57.130 ± 0.95).

Conclusion: We concluded that adding of the [6] -Gingerol as an antioxidant to the freezing and thawing medium can improve the sperm cryopreservation condition.

Keywords: Antioxidant, Cryopreservation, [6]-Gingerol, Sperm

Ethics and Reproductive Health

P-106: Psychological Variables Effects on The Success of Fertility Treatment

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Background: This study aimed to examine the effectiveness of mindfulness based stress reduction on success of ICSI/ IVF treatment, infertility stress, psychological symptoms and quality of life in infertile women.

Materials and Methods: In this prospective study, a total of 130 infertile women (referred to Royan Institute in Tehran) were studied during their first IVF treatment. All subjects completed questionnaires of demographic, Depression Anxiety and Stress Scale (DASS-21), Brief Symptom Inventory, Fertility Problem Inventory and Fertility Quality of Life. The patients were randomly and equally divided in two groups of intervention and control. The data were analyzed using both adjusted and unadjusted approaches. In the unadjusted methods, Chi-square test was used to investigate any association between the study group and categorical variables. Independent samples and paired t-tests were utilized to assess the continuous variables mean difference between the groups and within the time, respectively. Since the data was collected in two different time points over the study duration, generalized estimating equation (GEE) approach was used to assess the adjusted effect of intervention and time on the psychological factors. Two different models were fitted; first the psychological responses were assessed by intervention, time and the interaction between time and intervention while in the second model the intervention and time variables were adjusted for education, number for abortions, type of infertility, marriage duration, infertility duration, age and cause of infertility.

Results: A total of 130 infertile patients were assessed. The results showed that among all the psychometric variables measured by the research tools, global stress which was calculated using the sums for all five subscales scores, subscales scores of global stress, total FertiQol score and social subscales of Core FertiQol were significant predictors of successful IVF/ICSI. Applying a negative binomial regression, it was found that for

one score increase in global stress and total FertiQol scores the incidence of successful IVF/ICSI decreases by a rate of 3%. The patients GSI score was the same before the intervention ($P=0.521$) and it decreased after the intervention both in within and between groups comparisons ($P<0.001$). After the intervention, the PST score in the intervention group was significantly higher than the control group ($P<0.001$). The PSDI score reduced in both groups and it was lower among patients in the intervention group ($P<0.001$). The stress, anxiety and depression decreased significantly just in the intervention group. In contrast to social concern, other dimensions reduced significantly in the intervention group. After adjusting for demographic/infertility variables, the effects of intervention, time and their interaction on psychological variables were not significant. According to the results of adjusted estimations (Model 2), the GSI score increased in the control group while decreased in the intervention. The PST score decreased in the control group and increased in the intervention group. The PSDI scores decrement was higher in the intervention group than control group. The mean decrease score of depression was 3.608 ($P=0.007$). Similar to unadjusted results, the GEE showed that social ($P=0.380$) and sexual concern ($P=0.505$) was not influenced by the intervention. In contrast, the mean decrease in relationship, rejection and need for parents concern in the intervention group was 3.451 ($P=0.043$), 8.824 ($P<0.001$), and 16.412 ($P<0.001$), respectively.

Conclusion: The present study suggests that MBSR is efficacious, both in reducing psychological distress and improving clinical pregnancy rates.

Keywords: Global Stress, MBSR, IVF/ICSI Success, FertiQol

P-107: Metformin Attenuates Cognitive Impairment and Anxiety in Ovariectomized Mice

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Background: Estrogen regulates many processes in the brain such as synaptic formation, learning, and memory. Empirical evidence shows that there is a correlation among menopause, memory impairment, and anxiety due to Estrogen deficiency. Regarding the neuroprotective and anti-inflammatory property of Metformin (Met), the present study was designed to evaluate the effects of Met on tactile learning and anxiety in ovariectomized (OVX) mice.

Materials and Methods: For this purpose, Met (7 and 15 mg/kg/p.o.), was administrated daily in OVX mice for 21 days. Tactile learning and anxiety like-behavior were evaluated by novel object recognition task and Elevated Plus-maze, respectively.

Results: Our findings showed that Met prevented from the deleterious effects of ovariectomy on learning memory (7 and 15 mg/kg). We also found the improvement in anxiety by increasing Open Arm Time (OAT%) and Open Arm Entries (OAE%) in OVX animals treated with Met at the both doses compared to the OVX group.

Conclusion: Collectively, the results of present study suggest that Met is a convenient choice in menopause for amelioration of memory dysfunction.

Keywords: Ovariectomy, Metformin, Learning Memory, Anxiety Like-Behavior

P-108: The Preventive and Curing Effects of Ferulic Acid on Diabetes and Pituitary-Gonad Axis Hormones in Streptozotocin-Induced Diabetic Male Rats

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Background: Diabetes mellitus is a common metabolic disease which significantly affects the reproductive system and one of the complications of this disease is decreasing testosterone levels in serum. Some studies indicated that medicinal plants may improve reproductive system function in diabetes. This study investigated preventive and therapeutic effect of ferulic acid on diabetes and pituitary-gonad axis hormones in streptozotocin-induced diabetic male rats.

Materials and Methods: In this study, 24 male Wistar rats (weighing 235 ± 25) were divided into 4 groups ($n=6$): 1- normal group 2- diabetic group 3- treatment group via ferulic acid 4- preventive group of ferulic acid (received ferulic acid for 10 days prior to the injection of STZ) 5- group to received ferulic acid until the end of the period. In this study, diabetes was induced by streptozotocin (40 mg/kg body weight i.p). ferulic acid was injected (10 mg/kg). The serum glucose levels were assessed via enzymatic colorimetric method and insulin, testosterone, LH and FSH levels in serum were assessed via Chemiluminescence method.

Results: Induction of diabetes increased glucose levels and reduced insulin levels in serum and administration ferulic acid in the treatment groups, reduced blood glucose concentrations and increased insulin levels. Moreover induction of diabetes reduced the levels of testosterone. Besides ferulic acid increased testosterone in the normal groups. Ferulic acid had no significant effect on the LH and FSH levels.

Conclusion: Ferulic acid may decrease blood glucose levels in STZ induced diabetic rats and ferulic acid may preventive effects in the induction of diabetes by STZ. Ferulic acid may increase testosterone levels in preventive and normal mode and in normal groups these agents may androgenic properties.

Keywords: Ferulic Acid, Testosterone, LH, FSH

P-109: Paternal Lifestyle: How Bad (Harmful) Are Trans Fatty Acids for Sperm Quality of Male Offspring

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Background: It has been demonstrated that paternal diet can effect on the metabolic status of the offspring. In the current study we tried to investigate the influence of paternal trans fatty acids diet on sperm quality of rat offspring. For this respect, 20 adult male Wistar rats (5 in each groups) were included to the study which their fathers were fed for 60 days in the following

four diet groups: (C) control diet (CT) control diet with trans fatty acid (E) a diet containing vitamin E and (ET) a diet containing vitamin E and trans fatty acid.

Materials and Methods: Epididymal tissues were collected from male offspring rats to analyze sperm parameters by using CASA computer analysis. Data were statistically analyzed using one-way ANOVA.

Results: There were significant differences in concentration, progressive and total motility of sperm parameters among groups. Accordingly to Duncan's test, sperm concentration in group with vitamin E intake was significantly higher than other groups ($P < 0.05$). Furthermore, the mean of progressive and total motility of sperms in group ETH and CTH was significantly lower than other groups.

Conclusion: In this animal model, consumption of paternal trans fatty acid had negative effects on normal spermatogenesis in male offspring, thus the quality and quantity of sperm were decreased. Also, contrary to our expectations, vitamin E supplement could not neutralize enough the negative effect of trans fatty acids in this regard.

Keywords: Paternal Diet, Trans Fatty Acid, Vitamin E, Sperm

P-110: Survey on Effect of Selenium as An Antioxidant on Different Sperm Parameters in Varicocele Rats

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Background: Varicocele is an abnormal enlargement of the pampiniform venous plexus and blood blockage in the spermatic cord. Among major effects on male fertility is oxidative stress and metabolites re ex to testicles, which in turn have a significant part in producing free radicals [reactive oxygen species (=ROS)]. As a micronutrient, selenium has an antioxidant characteristic to normal spermatogenesis and combats oxidative stress. The present study aimed at examining selenium effects, as an antioxidant element) on sperm parameters (sperm count, motility, morphology) in varicocele rats.

Materials and Methods: This study was a case-control. It was done in 2017 in the Qazvin University of Medical Sciences on 24 male Rats that were divided randomly to four 6-subject groups of control, sham, varicocele, and treated varicocele. A daily 0.2-mg selenium diet was injected intraperitoneally to the treated varicocele group. After four weeks, sperm indices were examined among all the groups and the data was analyzed by one-way ANOVA.

Results: All the indices in this study (sperm count, motility and morphology) had a significant decline in the varicocele group compared to the control group ($P < 0.05$). Results also indicated an effect of selenium as an antioxidant on the number of sperm as well as their motility. This led to improvement of these parameters having no effects on sperm morphology among varicocele rats.

Conclusion: Results showed that, as an antioxidant, selenium eliminated free radicals in varicocele rats indicating its effective-

ness on varicocele-dependent infertility.

Keywords: Varicocele, Sperm Motility, Rat, Sperm Count, Sperm Morphology

P-111: Oxidative Stress Is Induced by Lithium Chloride in Human Sperm Motility and Viability-Protective Effects of Silymarin

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Background: Lithium is an environmental pollutant which is used in pharmacy industry. Lithium chloride exerts harmful effects on male reproductive system and human sperm by induction of oxidative stress. Silymerin as a potent antioxidant extracted from Silybum marianum can reduce the oxidative stress. The aim of this study was to investigate if silymarin can protect the harmful effects of lithium chloride on human sperm motility, viability, total antioxidant capacity and lipid peroxidation.

Materials and Methods: In this study, high quality spermatozoa were used. The samples were washed by human tubal fluid containing bovine serum albumin. The sperm suspensions were divided into 5 groups ($2 \times [10]^7$ spermatozoa per group). 1. Spermatozoa at 0 hour, 2. Spermatozoa incubated for 3 hours (control), 3. Spermatozoa treated with lithium chloride (0.5mM) for 3 hours, 4. Spermatozoa treated with silymerin (0.1mM) for 3 hours and 5. Spermatozoa treated with silymarin and lithium chloride for 3 hours. Sperm motility was performed according to World Health Organization (WHO) guidelines and eosin-nigrosin staining, while malondialdehyde (MAD) and ferric reducing antioxidant power (FRAP) were assessed to investigate lipid peroxidation and total antioxidant capacity respectively. The results were analyzed using one-way ANOVA and $P < 0.05$ was considered significant.

Results: Lithium chloride reduced a significant ($P < 0.000$) in percentage of motility, viability and total antioxidant capacity, while increased ($P < 0.001$) the amount of MAD compared to the control group. In the silymerin+lithium chloride group, silymarin could significantly ($P < 0.001$) reverses the toxic effect of lithium chloride on these parameters, when compared to the lithium chloride group. The application of silymarin alone significantly ($P < 0.001$) increased motility, viability, FRAP and decreases the amount of MAD as compared to the control group.

Conclusion: Lithium chloride by inducing oxidative stress exerts toxic effects on motility, viability, FRAP and MAD and silymarin as a potent antioxidant compensate the adverse effects of lithium chloride.

Keywords: Lithium Chloride, Silymerin, Motility, Viability, Human Sperm

P-112: Psychological Disorders and the Sexual Quality of Life among Infertile Women with and without Polycystic Ovary Syndrome Seeking Assisted Reproduction Treatment

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Background: Polycystic ovary syndrome (PCOS) is a serious

and frequent endocrine disorder in Women. Common features of PCOS, including hyperandrogenism (elevated Male hormones), ovarian dysfunction and obesity, can be highly distressing. Psychological factors can play a role in PCOS. The studies showed Polycystic ovary syndrome reduces sexual satisfaction in women. Depression, anxiety and many psychological symptoms appear to be common in women with PCOS. We investigated the effects of PCOS on sexual quality of life and psychological disorders among infertile women.

Materials and Methods: A cross-sectional survey was conducted on 300 (150 PCOS and 150 non-PCOS) infertile women referred to the Royan Institute, a referral infertility clinic in Tehran, the capital of Iran, between 2015 and 2016. The sampling method was convenient. Women responded to the Symptom Checklist-90 (SCL90) and sexual quality of life female (SQOL-F) questionnaires. Data were analyzed with SPSS software using Independent sample t test.

Results: The results showed that there is no significant difference between subscales of SQOL-F and all nine subscales of SCL90 including Hostility, Anxiety, Obsessive-compulsive, interspersed sensitivity somatization, psychoticism, paranoid ideation, depression and phobic anxiety among infertile women with or without polycystic ovary syndrome ($P < 0.001$).

Conclusion: According to the results, it can be said that although polycystic ovary syndrome may have a negative effect in different aspects of a woman who suffers from, it cannot significantly reduce the sexual quality of life and psychological disorders among infertile women.

Keywords: Psychological Disorders, Sexual Quality of Life-Female, Polycystic Ovary Syndrome, Infertile

P-113: Predictive Factors for Embryo Implantation Success Rate: An Application of Data Mining

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Background: In spite of recent improvements in infertility treatment, there is no significant increase in pregnancy rates. While the cost and complex process of assisted reproductive technologies (ART) are the main challenging issues. The aim of this study is to predict key factors that could be helpful in select the best embryos to transfer.

Materials and Methods: In this study, information of 447 patients and 1154 transferred embryos at day 3, 4 and 5 were collected. Dataset contains 63 variables and a class label, indicating positive and negative implantation outcomes. The relative predictive values of clinical features were assessed using ranking-based algorithms such as Gain ratio and Gini Index in Orange data mining software.

Results: The results revealed that, the quality of transferred embryos is the most important predictive factor among examined IVF/ICSI features. Our findings show that the FSH/HMG dosage for ovulation stimulation is the second most important factor in Implantation outcomes. Number of MII quality oocytes and number of blastomeres are other high-ranked features for prediction of the embryo implantation success rate.

Conclusion: Elicited decision rules from data mining ranking algorithms offer a clinical decision support tool for selecting the best embryos that lead to improvement in ART success rates.

Keywords: Assisted Reproductive Technologies, Data Mining, Ranking Algorithms, Clinical Decision Support

P-114: Comparison of Health Related Quality of Life between Normal-Weight and Women with Excess Body Weight

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Background: Excess body weight is one of the most serious public health challenges in Iran. Some studies demonstrated that increasing body weight worsened all aspect of health related quality of life (HRQOL); these studies were mostly conducted in western countries. In contrast, others showed that obesity is adversely associated with physical but not mental HRQOL. The aim of this study is to compare HRQOL between normal weight and women with excess body weight.

Materials and Methods: Participants were selected from among women aged 18-40 years, attending gynecologic centers affiliated to Islamic Azad University of Medical Sciences in Tehran for their annual gynecological exam. Exclusion criteria included pregnancy, breastfeeding, chronic disease or cancer. None of the women had history of depression or anxiety. Obesity was defined based on body mass index (BMI) and the Iranian version of the short form health survey 36 (SF 36), used for assessing HRQOL. BMI levels were classified as follows: BMI 18-24.9 kg/m² as normal, and BMI ≥ 25 kg/m² as overweight or obese. Data were analyzed by ANOVA and MANOVA. The relationship between BMI and domains score of SF36 was examined using the Pearson correlation test.

Results: 428 women including 228 overweight or obese and 200 normal weight completed study. Mean BMI was 26.5 ± 3.2 kg/m². After adjusting for age, parity, marital status and educational levels, results showed that the normal weight women had significantly higher bodily pain (BP) (89.4 ± 2.3 vs. 70.2 ± 2.4), physical functioning (PF) (82.4 ± 2.4 vs. 73.5 ± 1.5), physical component summary scales (PCS) (83.3 ± 1.3 vs. 70.9 ± 1.7) scores than overweight or obese ones ($P < 0.05$). The scores in other physical and mental aspects of HRQOL didn't differ between two BMI categories. There was a significant correlation between BMI and BP, $r = -0.47$, PF, $r = -0.2$, PCS, $r = -0.32$, ($P < 0.001$).

Conclusion: The results of study showed negative impact of excess body weight on physical aspect of HRQOL.

Keywords: Health-Related Quality of Life, Body Mass Index, Obesity, Overweight

Female Infertility

P-115: The Relationship between Endometrial Length in Transvaginal Sonography and IVF Adaption in Infertile Patients of Royan Institute

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Background: The outcome of IVF depends on the quality of the fetus and the parameters of the endometrium. Various studies have been done about success of IVF treatment, but no stud-

ies on the length of the endometrium (from the Fundus to the Int. Os.) with ultrasound have been conducted. The aim of this study was to determine the relationship between endometrium length at daytime HCG injection (Human gonadotropin hormone) and IVF adaption.

Materials and Methods: This retrospective cohort study was conducted in 2017 on eligible infertile patients of the Royan Institute of Tehran. The endometrial length was measured by ultrasound sonography with the Madison and alpha 10 devices. In the treatment cycle, two groups [A (endometrial length less than or equal to 40 mm / day HCG injection) and B (endometrial length greater than 40 mm)] were examined. The results and treatment success of the groups were compared using Chi-square test in SPSS software.

Results: Of the total 166 cases, successful cases were 39.8 % (30.3% were in group A and 69.7% in group B). Out of 66 cases in group A, 33.3% had successful IVF and 69.7% had unsuccessful treatment. Out of 100 cases in group B, 46% had successful treatment and 54% had unsuccessful treatment. This difference was statistically significant ($P=0.31$).

Conclusion: Considering the psychological, physical and economic burden of infertility treatment and the positive relationship between endometrial length and the success of IVF treatment, more specialized investigation is suggested in this context.

Keywords: IVF, Endometrium Length, Transvaginal Sonography

P-116: Effects of Phytoestrogen Genistein on Serum Interleukin-6 Levels in Model of Polycystic Ovary Syndrome Induced with Esteradiol Valerate

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Background: Polycystic ovary syndrome (PCOS) is the most common reason for infertility due to lack of ovulation among women in reproduction age that involves 10-15 percent of women. According to Rotterdam criteria, about 15.2 % of Iranian women suffer PCOS. Studies suggest that low-grade chronic inflammation could be concern in pathobiology of this incident disorder. Interleukin-6 (IL-6), a pleiotropic cytokine, plays an important role in the endocrine system, especially as related to ovarian maturation and the processes of fertilization and implantation. IL-6 has also been shown to modulate ovarian development and function. Therefore, IL-6 may be a key mediator of low-grade chronic inflammation in PCOS. Genistein (4',5,7-trihydroxyisoflavone) is the principal isoflavone found predominantly in soybeans, and has attracted considerable attention due to its potential effects on some of the degenerative diseases, such as cardiovascular disease, osteoporosis, and hormone-related cancers. Interest in genistein as a potential therapeutic agent for inflammation has recently risen, as studies have shown that genistein exerts evident anti-inflammatory properties. However, detailed molecular mechanisms of the anti-inflammatory effects of genistein are still elusive. Now, to care the positive aspects of phytoestrogen Genistein effect on increasing the anti-oxidant and anti-inflammatory in body and furthermore high incidence, lack of definite cure for PCOS and side effects of exist cure methods, reveal the purpose of present

investigation.

Materials and Methods: In this study, the syndrome induced by muscular injection of 40 mg/kg Estradiol Valerate to 32 female Wistar rats, weighing 170-180 g. Control group receive no injection. After 60 days the animals divided to control, PCOS and PCOS treated with Genistein (2 and 5 mg/kg) groups. After 21 days intraperitoneal treatment of genistein, the ovaries of all groups histologically studied. Serum interleukin 6 levels were detected by the ELISA kit. Data were analyzed using SPSS via one-way analysis of variance (ANOVA) and $P<0.05$ was considered statistically significant.

Results: There was a signification polycystic improvement in ovaries treated with high concentration of Genistein in comparison with PCOs group and also IL-6 level decrease, thickness of the theca layer decrease, reduction of cystic follicular number, corpus luteum increase and granulosa layer increase were found, that could be a sign of renewed ovulation.

Conclusion: Genistein could decrease PCOS histological signs by decreasing the serum levels of inflammatory cytokines that lead to ovulation rate reduction, and have reparation effects on ovary tissue. It is possible that, this ability is because of Genistein antioxidant and anti-inflammatory effects that motivate reduction of cysts number and natural development of follicles.

Keywords: Genistein, Interleukin-6, Infertility, Polycystic Ovary Syndrome, Inflammation

P-117: Morphometric and Morphology Oocytes after *In Vitro* Maturation in Patient Women with Polycystic Ovarian Syndrome

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Background: The application of (IVM) oocytes to help clinical infertility treatment still remains poor because of developmental competence of oocytes decrease after IVM. Moreover, oocyte maturation and fertilization rate are low *in vitro* matured compared with *in vivo*-matured. The aim of this study was to comparison of morphometric and morphology oocytes after IVM in patient women with polycystic ovarian syndrome (PCOS).

Materials and Methods: 32 women; 20-35 year-old, undergoing controlled ovarian stimulation for ICSI/IVF protocol. The immature oocytes (n=108) were divided into two groups: the first group in normal women (n=54); and the second group in women with PCOS (n=54), then directly matured *in vitro*. After 24-48 h of incubation, the oocyte maturation rate, morphometric and morphology characteristics were assessed using imaging by inverted microscope and were compared.

Results: There were significant differences in the maturity oocytes after IVM between normal women and patient women with PCOS ($P<0.05$). Moreover, the morphometric assessment revealed that there were no significant differences in the mean oocytes of total diameters (μm) (zona thickness (ZPT) + perivitelline space width (PVS) + cytoplasm (CD) between normal women and women with PCOS, 156.3 ± 6.8 and 137.7 ± 9.9 , respectively ($P>0.05$). Evaluation of morphological oocytes showed that morphological abnormalities include vacuolization and ooplasm granulation were higher than in PCOS women when compared with normal women ($P<0.05$).

Conclusion: IVM of oocytes in normal women was more successful than those in women with PCOS.

Keywords: PCOS, IVM, Human Oocytes

P-118: Altered Expression of ADAMTS-1, ADAMTS-4 and Nuclear Progesterone Receptors Associated with Impaired Oocyte Maturity in PCOS Patients

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Background: A series of proteinases, such as the matrix metalloproteinases, a Disintegrin-like and Metalloproteinase with Thrombospondin type-1 motif (ADAMTS) proteinases, have very critical roles in damage and repair of extracellular matrix (ECM) processes (remodeling). The aim of this study was to investigate changes of ADAMTS-1 and ADAMTS-4 gene expression in cumulus cells of PCOS and their association with progesterone receptors expression and oocyte maturity.

Materials and Methods: In this study, 74 infertile women 18-40 years old who underwent oocyte recovery at an IVF clinic were recruited; 37 PCOS patient and 37 infertile women with normal ovulatory function as the control group. After approval of Hamadan University of Medical Sciences ethics committee and written informed consent of the patients, cumulus cells (CCs) were obtained from women undergoing oocyte retrieval and categorized according to the oocyte nuclear maturation stage: GV or MII (metaphase II stage). Total RNA was extracted and reverse transcription was performed. Gene expression of ADAMTS-1 and ADAMTS-4 was determined by quantitative real-time PCR method (q-PCR).

Results: Our data revealed that a decreased expression levels of ADAMTS-1 and ADAMTS-4 in the CCs of PCOS women compared to the healthy women with normal ovarian function ($P=0.02$ and $P=0.01$ respectively). Furthermore, we observed increased expression levels of ADAMTS-1 and ADAMTS-4 in the CCs of mature oocytes compared to the GV oocytes ($P=0.02$ and 0.01 respectively). In this study, we observed a strong positive correlation between the expression of progesterone receptor A with ADAMTS-1 and ADAMTS-4 ($r=0.71$, $P=0.0001$, and $r=0.58$, $P=0.0001$ respectively). The sensitivity and specificity of ADAMTS-1 were found to be 90% and 67%, respectively [(an area under the ROC curve (AUC) of 0.8)].

Conclusion: ADAMTS-1 and ADAMTS-4 express in GV oocytes with lower levels in mature oocytes and might be an important manifestation in gonadotropin-stimulated patients which could influence oocyte maturation and can be considered as a potential diagnostic biomarker.

Keywords: ADAMTS-1, ADAMTS-4, PCOS, Cumulus Cells, Oocyte Maturity

P-119: The Incidence of Gestational Trophoblastic Disease following Assisted Reproductive Technology Cycles

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Background: Gestational trophoblastic disease (GTD) is a heterogeneous group of diseases characterized by excessive proliferating trophoblastic tissues. The presence of additional paternal genome (dispermia) and maternal chromosome loss appear to be the critical role in the pathogenesis of Hydatidiform mole. Epidemiological studies have shown the prevalence of GTD is impressed geographic variation distribution. HM occurs in 3-7 per 1000 spontaneous pregnancies in Iran, however its prevalence after assisted reproductive technology (ART) cycles have not been reported. This study aimed to estimated GTD frequency subsequently ART cycles.

Materials and Methods: This retrospective study was evaluated all patients with a diagnosis of GTD subsequently ART cycles at Royan institute from 2008-2015 years. HM prevalence was estimated per embryo transfer (ET), clinical pregnancy (CP) and live birth (LB). Also, previous related risk factors such as maternal age, blood group, miscarriage history, familiar GTD history and paternal age were evaluated by questioner form. HM confirmed by serial β hCG titer, ultrasonography and histopathology assessment of the evacuated uterine contents.

Results: Twelve GTDs were identified during the study included 10 HM and 2 Gestational trophoblastic neoplasias (GTN) which one case has metastasized to the pulmonary. The incidence of GTD was estimated 0.31% in 37841 ET, 0.11% in 10813 CP (between 2008-2015 years) and 0.129% in 3522 normal LB (between 2012-2015 years). The mean of female and her partner's age were 26.66 ± 1.32 and 34.16 ± 1.3 years, respectively. Patients have no history of familial GTD and miscarriage whereas two cases (16.7%) have previous spontaneous abortion. The most common blood group was O in women.

Conclusion: For the first time, we described prevalence of GTDs following ART cycles that this frequency was 1:1000 CP, LB and 3:1000 ET. This result is comparable to 3-7 per 1000 spontaneous pregnancies in Iran. Based on present study, proportion of GTDs in normal pregnancy was 1-3 times more than this frequency after ART in Iran.

Keywords: Assisted Reproductive Technology, Gestational Trophoblastic Disease, Hydatidiform Mole, Prevalence

P-120: The Anti-Inflammatory Effect of Flavonoid Apigenin on Ovarian Histological Changes in Polycystic Ovary Syndrome Rat Model

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in the reproductive age female around the world. Chronic low-grade inflammation has been suggested to play an important role in the the pathogenesis and development of this syndrome. Apigenin is a non-toxic natural flavonoid that is abundantly present in common fruits and vegetables. It has been reported that apigenin has various beneficial health effects such as anti-inflammation and chemo-

prevention. Multiple studies have shown that inflammation is an important risk factor for atherosclerosis, diabetes, PCOS, sepsis, various liver diseases, and other metabolic diseases. Regarding the antioxidant and anti-inflammatory properties of flavonoid Apigenin, this study was conducted to determine the effect of Apigenin on morphology of ovarian tissue in pcos rat model.

Materials and Methods: In this study, the syndrome induced by muscular injection of 40 mg/kg Estradiol Valerate to 32 female Wistar rats, weighing 170-180 g. After 60 days the animal divided to control, pcos and pcos treated with Apigenin (20 and 40 mg / kg by injection for 21 days). At the end of study, the rat ovaries were evaluated histologically.

Results: Histological studies showed significant increases in the number of primordial, primary, pre-antral and cystic follicles in comparison with the control group ($P < 0.05$). Measurement of granulosa, Theca, number and diameter of different cysts and follicles showed significant improvement in polycystic ovary in rats treated with Apigenin.

Conclusion: These findings indicate that Apigenin have protective effects on polycystic ovary syndrome maybe due to its antioxidant and anti-inflammatory properties.

Keywords: Polycystic Syndrome Ovary, Apigenin, Ovary

P-121: Mechanisms Involved in Oocyte Maturation and Embryo Development in PCOS: Bioinformatics Analysis

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Background: Polycystic ovary syndrome (PCOS), an multifactorial endocrine disorder that involved 5-10% of reproductive age women and responsible for 75% of lack ovulation infertility. The prevalence of syndrome is more than 6% in Iran. The risk of miscarriage in early pregnancy and premature birth increased in women with polycystic ovary syndrome. This study we investigate mechanisms involved in polycystic ovary syndrome.

Materials and Methods: By studying numerous articles were extracted about 447 (up-regulated or down-regulated) proteins that were significantly involved in polycystic ovary syndrome. For network construction and its analysis use Cytoscape software. Gene ontology analysis for whole proteins was performed on Bingo Cytoscape app.

Results: SIRT1 is known proteins that showed high degree among the 447 proteins that were involved in mechanisms of PCOS. This protein is a NAD-dependent protein deacetylase sirtuin-1. The most significant proteins obtained of gene ontology were showed roles in protein folding, cell aging, tricarboxylic cycle, glycolysis and metabolic process in PCOS.

Conclusion: SIRT1 is a NAD-dependent protein deacetylase sirtuin-1 that can modulate chromatin function through deacetylation of histones and can promote alterations in the methylation of histones and DNA, leading to transcriptional repression. Glycolysis and tricarboxylic cycle are pathways associated with glucose metabolism, disturbances in these pathways cause diabetes in women with polycystic ovary syndrome. MDH1,

SOD1, SOD2 and PHB2 proteins plays a role in ovary aging. Thses pathways prevent oocyte maturation in pcos women and cause infertility in this group of women. Therefore, recognizing these molecules can be effective in improving fertility in PCOS women.

Keywords: PCOS, Proteomic, Oocyte Maturation, Gene Ontology

P-122: GCSF May Improve Pregnancy Outcome in Blastocyst Embryo Transfer Patients with A History of Unexplained Implantation Failure and Normal Endometrium: A Randomized Control Trial

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Background: The family of colony-stimulating factors (CSF) plays a pivotal role in the early dialogue between mother and embryo. The aim of this study was to evaluate the effects of the single dose G-CSF injection in an unexplained group of patients with repeated implantation failures with normal endometrium in whom embryo transfer has been done in blastocyst stage.

Materials and Methods: This randomized control trial study was performed on 52 infertile women who referred to the clinic with the history of more than three previous IVF/ICSI-ET failures. All patients were stimulated with standard long protocol. All embryos were transferred on day five in blastocyst stage in both groups. The treated group received 300 µg (0.5ml) recombinant human GCSF subcutaneously injected 30 minutes before blastocyst embryo transfer. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 16.0

Results: G-CSF treated group showed higher clinical pregnancy rate (56.2%) in comparison with control group (40.0%) but it was not statistically significant ($P = 0.09$, chi-square). Although the rate of live birth rate in G-CSF group was higher than control group (53.1% vs. 35.0%) but there was positive but not statistically significant difference in the overall live birth rate between the two groups ($P = 0.10$, t test).

Conclusion: Our result demonstrates that pregnancy outcome was better in women with repeated IVF failure who are treated with G-CSF.

Keywords: Granulocyte Colony-Stimulating Factor, Embryo Implantation, Pregnancy Rates, Randomized Controlled Trial

P-123: Expression of Tumor Necrosis Factor-Alpha and Estrogen Receptors Gene in Endometrial Tissue Riosis

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Background: Endometriosis is classically defined as the presence of functional endometrial glands and stroma outside the uterine cavity. Physiological changes during menstrual cycle cause the endometrium and endometriosis to develop specific kind of tissues, especially in regard to the gene expression profiles. Alterations in estrogen-mediated cellular signaling play an essential role in the pathogenesis of endometriosis. ER β interacts with cellular apoptotic machinery in the cytoplasm to inhibit Tumor necrosis factor (TNF- α)-induced apoptosis. TNF- α is a major cytokine involved in inflammatory and immune function. Our aim in this study was to determine the expression of TNF α and ER α , ER β in endometriosis.

Materials and Methods: With the usage of RT-PCR, we researched the expression of as TNF α and ER α , ER β reliable reference genes in eutopic and ectopic endometrial tissue specimens obtained during standard surgery of women of reproductive age. Stability of expression level was analyzed by the most universal MS excel.

Results: During this study, changes in the expression of TNF α and ER α , ER β were observed between normal and endometriosis groups in various phases of menstruation.

Conclusion: In RT-PCR based analyses of gene expression level in eutopic and ectopic endometrium, we strongly recommend that a difference of TNF- α and ER α , ER β genes are to be used.

Keywords: Endometriosis, TNF α , Apoptosis, ER α , ER β

P-124: Mthfr Gene 677 C/T Polymorphism Increased The Risk of Preterm Labor in Iranian Pregnant Women

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Background: The MTHFR gene known to be involved in the homocysteine-methionine pathway. Deficiency of MTHFR activity may cause hyperhomocysteinemia which results in adverse pregnancy outcomes. Since, we analyzed the genetic association of MTHFR gene 677 C/T polymorphism with preterm labor in Iranian pregnant women.

Materials and Methods: This study performed in a total of 80 Iranian pregnant women (40 preterm labor and 40 normal labor controls). Study of MTHFR gene 677 C/T polymorphism frequency between the case and the control were assessed. Using chi-square and logistic regression tests for analysis. The relevant risk of preterm delivery was represented by odds ratios (ORs) with 95% confidence intervals (95% cIs).

Results: Based on the results of this study, C/T gene type frequency of MTHFR gene C677T polymorphism was higher in cases than the controls (P=0.003, OR=0.96, 95%, CI=0.26-3.64) whereas, no significant correlation was found between CC (P=0.46) and TT (P= 0.37) gene types with preterm labor. In addition, there was no significant relationship between T allele and preterm labor.

Conclusion: The results of this study demonstrated that gene type CT of MTHFR C677T polymorphism might make preterm delivery risk rise in Iranian women.

Keywords: Preterm Labor, MTHFR, Pregnancy

P-125: In Vitro Model Three-Dimensional Culture for The Study of Human Implantation

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Background: Implantation involves coordinated interaction between the embryo and the receptive endometrium. Implantation failure is one of the major causes of subfertility. However, the detail mechanism of implantation is still not very clear. Studying human embryo implantation is challenging since *in vivo* experiments are impractical and unethical, and studies in animal models do not always translate well to humans. Thus, it is necessary to develop an *in vitro* experimental model that can better reflect the *in vivo* situation for studying implantation.

Materials and Methods: *In vitro* novel three-dimensional (3-D) endometrium cultures were constructed with fibrin-agarose as matrix scaffold. Endometrial biopsies were obtained from healthy, fertile women on cycle day LH+4 and stromal and epithelial cells were isolated. Stromal and epithelial cells were characterized by flow cytometry and immunocytochemistry. Human endometrial stromal cells embedded in a mixture of fibrin-collagen I that covered with a layer of epithelial cells and cultured until confluence. Healthy, viable human embryos were placed on cultures. Two days later, the cultures were tested for the development and attachment of embryos. The biological growth, live and dead staining, MTT assay and haematoxylin and eosin staining of cultured cells were performed.

Results: Histological staining showed that epithelial cells were organized in a polarized columnar epithelium on the apical surface gel. A polymer gel that not only supports cell growth but handily transforms from gel to liquid After a few days. Cell viability was evaluated using live/dead and MTT assay showed that the 3D cultures were not toxic for the cell. Cell viability tests showed that stromal and epithelial cells developed and survived and were compatible with scaffold.

Conclusion: In this study, an *in vitro*-three dimensional embryo-endometrial cell culture model to investigate the human embryo implantation process was established that appeared to imitate of the normal human endometrium.

Keywords: Three-Dimensional Culture, Implantation, Human Endometrium, Model

P-126: Breastfeeding and Risk of Endometriosis

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Background: Endometriosis is the benign proliferation of functioning endometrial glands and stroma, located outside of the uterine cavity. It is diagnosed by laparoscopic observation of lesions, nodules, or cysts on the pelvic peritoneum or the pelvic organs, and is one of the most common diseases in gynecology field, as well as a source of an exorbitant economic burden in public health field. Endometriosis could be considered as an epigenetic, hormonal regulated disease which is progesterone resistance, and estrogens promote perilesional angiogenesis and neo-innervation and allow endometriotic foci to growth. The nutritional benefits of breastfeeding for infants and the metabolic benefits for the mother are well known. The World Health Organization recommend that women breastfeed each child for at least 12 months with six months of exclusive breastfeeding (breastfeeding without the introduction of solid food or formula). The aim of this study is to investigate the association between breastfeeding and risk of endometriosis among women.

Materials and Methods: The case-control study was conducted between May 2016 and February 2017 on endometriosis women. Case group (n=78) women with a laparoscopically confirmed diagnosis of endometriosis and controls were 78 healthy women. Women reported duration of total breastfeeding, exclusive breastfeeding. All statistical analyses were performed by the SPSS software (version 20.0, SPSS, Chicago, IL, USA).

Results: Duration of total and exclusive breastfeeding was significantly associated with decreased risk of endometriosis. Among women who reported a lifetime total length of breastfeeding of less than one month. For every additional three months of total breastfeeding, women experienced a 6% lower risk of endometriosis (hazard ratio 0.82, 95% confidence interval 0.80 to 0.84; $P < 0.001$ for trend) and a 12% lower risk for every additional three months of exclusive breastfeeding (0.76, 0.71 to 0.80; $P < 0.001$ for trend). Women who breastfed for ≥ 6 months had a 30% reduced risk of endometriosis compared with women who never breastfed (0.50, 0.40 to 0.61).

Conclusion: Among women who experienced at least 6 months, breastfeeding was inversely associated with risk of endometriosis. This association was partially, but not fully, suggesting that breastfeeding could influence the risk of endometriosis.

Keywords: Breast Feeding, Endometriosis, Case-Control

P-127: Assisted Reproductive Technology: Predictive Factors for Gestational Diabetes Mellitus

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Background: Well-known predictors for development of diabetes in spontaneous pregnancy are maternal age, obesity, and

family history of diabetes. To date, studies concerning ART outcomes were unable to establish gestational diabetes mellitus (GDM) risk factors during ART procedures. It remains unclear which ART characteristic(s) is able to predict GDM in these subjects. Present study was performed to evaluate predictive factors for GDM in singleton pregnancy following assisted conception.

Materials and Methods: This nested case-control study was performed during October 2016 to June 2017. Pregnant women who conceived following ART procedures referred to infertility clinic were selected and categorized into GDM and non-GDM based on ADA/IAPDSG criteria. The study variables including age, educational status, and first-degree family history of chronic diseases, systolic and diastolic blood pressure, previous obstetric and perinatal outcomes, infertility history, and ART cycle characteristics were collected from medical records. Prediction model to develop GDM was employed by binary logistic regression analysis after adjustment for age and body mass index, family history of diabetes, and gravidity.

Results: In total, 270 women with singleton pregnancies (consisted of 135 GDM and 135 non-GDM women) conceived were studied. According to the final model, significant predictors of GDM were history of polycystic ovarian syndrome (PCOS), previous ovarian hyper-stimulation syndrome (OHSS) risk and progesterone injections. Administration of injectable progesterone during the first 10-12 weeks of pregnancy was associated with an approximately two-fold increased risk of developing GDM [odds ratio (OR) 2.28, 95% confidence interval (CI) 1.27-4.09] compared to vaginal progesterone. In addition, the regression analysis revealed that previous OHSS risk (OR 2.40, 95% CI 1.34-4.31) and history of PCOS (OR 2.76, 95% CI 1.26-6.06) were other most important predictors of GDM.

Conclusion: The route of progesterone administration, previous OHSS risk and history of PCOS seem to be putative risk factors for GDM in women conceived by ART. These findings could be considered in patients' consultation before ART and after achieving pregnancy.

Keywords: Gestational Diabetes Mellitus, Assisted Reproductive Technology, Predictive Factors

P-128: Comparison of Microdose Gonadotropin-Releasing Hormone Agonist Flare up and Flareup Protocol in Poor Responders Undergoing Intracytoplasmic Sperm Injection Cycles

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Background: In this study, an attempt was made to compare the two stimulation protocols of GnRH agonist flare-up (F) and microdose GnRH agonist flare-up (MF), used in patients who had a poor response to intracytoplasmic sperm injection cycle (ICSI).

Methods and Materials: In this randomized controlled trial study, which was conducted between September 2008 and May 2014, a total of 131 poor responder patients who underwent intracytoplasmic sperm injection cycles were included. Patients were excluded from the study if they had only one ovary, myoma ≥ 6 cm, azospermic partner, tumor or cyst > 13 mm, age > 42 . Patients were randomly assigned to one of the two groups

of MF which required the administration of buserelin at 80 µg, sc (n=66) and F protocol which required the administration of buserelin at 500 µg, sc (n=65). The primary outcome measures were clinical pregnancy and live birth rates. Statistical analysis was performed using SPSS software. In all tests, the significance level was considered less than 0.05.

Results: Both groups were comparable in regards to mean age, body mass index, type of infertility, infertility duration, basal hormone levels, gonadotropin type and history of surgery. Likewise, there were no significant differences with respect to cycle cancellation, the mean number of dominant follicles, achieved oocytes and MII oocytes, hormone levels on hCG day, number and quality of embryos transferred between groups. Additionally, number of stimulation days and endometrial thickness were significantly (P=0.032 and P=0.001 retrospectively) and gonadotropin dose was insignificantly (P=0.075) higher in MF group than F group. No statistically significant differences were found in rates of clinical pregnancy, implantation and miscarriage with MF regimen compared with F protocol. Live birth was significantly (P=0.036) higher in MF group than F group.

Conclusion: In conclusion, MF protocol seems to be superior to the F protocol but requires a higher dose of gonadotropins and longer duration of stimulation. Although, this would obviously result in differences in costs of money, this improvement is of probable clinical importance. Further prospective clinical trials on this topic are recommended.

Keywords: Microdose Gonadotropin-Releasing Hormone Agonist Flare up, GnRH Agonist Flare-up, Poor Ovarian Response, Intracytoplasmic Sperm Injection.

P-129: Troxerutin Affects Histological Structure of Ovary in Offspring of High Fat-Induced Obese Rats by Reduction of Apelin-13 Receptor Expression

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Background: Feeding and physical condition of mother can affect offsprings development. Previous studies indicated that obesity and high fat diet (HFD) feeding during pregnancy have deleterious consequences on reproductive functions of the offsprings. It is also indicated that apelin and its receptor play a critical role in human follicular development and pathogenesis of polycystic ovary syndrome. Troxerutin (TX) is a natural bioflavonoid which has many biological effects and improves obesity and its related metabolic parameters in HFD-treated mice. This study was conducted to evaluate the effects of the TX administration during pregnancy on histological structure of ovary, plasma level of apelin-13, and expression of its receptor in ovarian tissue of HFD fed rats offsprings.

Materials and Methods: In this study, 40 female 3 week-old wistar rats were randomly divided into the normal and HFD groups. Normal group received standard diet while HFD group received HFD diet for 8 weeks and then placed with normal male rats for copulation. After mating, pregnant animals of each group were divided into two subgroups including treated and non-treated with TX (150 mg/kg/day) by oral gavage until the end of pregnancy. Female offsprings of all groups selected and fed normal diet. Ninety days after birth (PND90), 32 off-

spring offsprings were euthanized and their blood samples and ovarian tissue were collected to serum concentration of apelin-13, histological structure of ovary and expression of apelin-13 receptor in ovarian tissue be evaluated. Serum concentration of apelin-13 detected by ELISA kit. Histological and histomorphometrical studies were carried out on paraffin embedding tissue section and H&E stained slides and expression of apelin-13 receptors in ovary tissue analyzed by real-time PCR. Finally, data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc ($\alpha=0.05$).

Results: Results showed that HFD reduced serum concentration of apelin-13 in comparison with control groups significantly (P<0.05) and TX had not significant effect (P>0.05). Real-time PCR analysis indicated that HFD increased expression of apelin-13 receptors in ovary tissue and TX could reduce it significantly (P<0.01). Microscopical studies revealed that HFD decreased percent of primary, secondary and antral follicles and increased atretic follicles significantly (P<0.05) while TX administration could improve these structures in the level of control groups.

Conclusion: Based on our results it can be concluded that administration of TX in high fat diet fed mothers during pregnancy can be effective as a suitable supplement to improve structural changes of ovary in their offsprings by reduction of apelin-13 receptor.

Keywords: Apelin-13 Receptor, High Fat Diet, Ovary, Pregnancy, Troxerutin

P-130: The Role of BAX, BCL-2 and Caspase3 Genes in Endometriotic Lesions and Normal Endometrial Tissues

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Background: Endometriosis is characterized by the presence of endometrial cells with capacity to avoid apoptosis outside the uterus. Apoptosis plays a fundamental role for the pathogenesis of endometriosis. The etiology and pathogenesis of endometriosis remain unclear. Apoptosis is regulated by several genes, especially those of the Bcl-2 gene family and caspase group genes. Different expression of these genes could contribute to the survival of regurgitating endometrial cells into the peritoneal cavity and the development of endometriosis. In this study we evaluate the different expression of BCL-2, BAX and caspase 3 in endometrial tissue on endometriosis lesion.

Materials and Methods: Tissue samples involving ectopic and eutopic lesions of patients with endometriosis were collected. To compare the relative expression of BCL-2, BAX and caspase 3 expression in normal endometrium during the menstrual cycle, we divided the biopsies into the following three groups: menstrual, proliferative, and secretory phases. Reverse Transcriptase PCR (RT-PCR) was performed on the prepared cDNA samples with the use of primers designed for BCL-2, BAX and caspase 3 expression.

Results: Different expression of BCL-2, BAX and caspase 3 expression was indicated in samples with endometriosis and normal endometrial tissue.

Conclusion: Women with endometriosis show decreased expression of apoptotic genes in endometriosis patient.

Keywords: BAX, BCL-2, Caspase 3, Endometriosis

P-131: Role of The Uterocervical Angle Assessment in Prediction of Preterm Birth in Singleton Pregnancies

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Background: Preterm birth is one of the most important problem in obstetrics and we could not predict its occurrence. The aim of this study is to find out whether the uterocervical angle (UCA) degree is related to predict the risk of SPTB (spontaneous preterm birth) < 34 weeks, without attention to cervical length.

Materials and Methods: In a pilot prospective Case-control study, 320 pregnant women that enter to study. For all of them transvaginal cervical length were measured at (16- 18) and (24-26) weeks and uterocervical angle were detected in both times. the including 30 mothers that had spontaneous preterm birth and gave birth before 34 weeks of gestation (SPTB group) and 290 who had delivery at term (control group). Images of UCA measurement at two stages in every patient, were compared.

Results: In the 24 -26 weeks Mean UCA increased in the (SPTB group) (105°) compared with the control group (95°). Mean UCA increased from the first to the second trimester (85° vs. 95°).

Conclusion: In the 24-26 weeks or in the second trimester Wider UCA is associated with SPTB. UCA measurement is a reproducible technique. UCA appears to increase from the first to the second trimester. It is consider that may be wider USA in first trimester or in other wise too widening USA from first to second trimester is an alarm for spontaneous preterm births. Prospective large studies, on measuring UCA in better condition, are considered necessary to perfectly evaluate the individuality of this parameter and its probability as an interpreter of spontaneous preterm birth in practical fields.

Keywords: Transvaginal Ultrasound, Cervical Length, Uterocervical Angle, Preterm Birth

P-132: The Relationship between Decrease in Cervical Length and Spontaneous Preterm Birth in Twin Pregnancies

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Background: The first aim is to find whether rate of shortening in cervical length is associated with preterm birth in twins.

Materials and Methods: This is a prospective cohort of serial transvaginal cervical length performed for twin pregnancies at

a single institution from 2013 through 2016. Women with diamniotic twin pregnancies who had transvaginal cervical length measurements at 18 and 24 weeks' gestation and follow up results were observed. Statistical methods were used to assess the association between the rate of alter in cervical length and the risk of premature birth < 34 weeks.

Results: Overall, 264 women had criteria for enter this work. The middling rate of alteration in length of cervix for patients with preterm birth < 34 weeks was 1.5 mm/wk vs. 0.9 mm/wk for who delivered ≥ 34 weeks. Women with fast alteration in transvaginal cervical length, ≥ 2 mm/wk, had 3 times higher the likelihood of spontaneous preterm birth as those with slower change.

Conclusion: Shortening in transvaginal cervical length in the 18 and 24 w is related with spontaneous preterm birth, so guidelines for sequential transvaginal cervical length checking can help the clinician to discover at-risk patients. ≥ 1.5 mm/wk decrease of cervical length identifies patients with prominent risk for spontaneous preterm birth under 34 weeks.

Keywords: Twins Gestation, Cervical Length

P-133: Assessment of Oxytocin Level, Glucose Metabolism Components and Cutoff Values for Oxytocin and Anti-Mullerian Hormone in Infertile PCOS Women

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Background: Comparing oxytocin level and some other parameters between infertile women with or without polycystic ovary syndrome (PCOS), to evaluate the correlation between oxytocin with anti-mullerian hormone (AMH), body mass index (BMI) and insulin resistance (IR).

Materials and Methods: This cross-sectional study was performed on 80 PCOS and 81 non-PCOS women as the control group. Oxytocin, various hormones, oral glucose tolerance test (OGTT) and homeostatic model assessment of insulin resistance (HOMA-IR) were compared between two groups. Correlations between parameters were assessed by the spearman's rank correlation coefficient. Cutoff values for oxytocin and AMH in PCOS were calculated by the ROC-Curve and DeLong method.

Results: The mean oxytocin level was statistically lower in the case group (P ≤ 0.001). The mean BMI, AMH, HOMA-IR, fasting insulin and insulin 2-hours after 75-g glucose was significantly higher in the PCOS group. Oxytocin was negatively correlated to AMH and also HOMA-IR. However the relationship between oxytocin and BMI was not significant. The calculated

cutoff value for oxytocin was 125 ng/L and for AMH was 3.6 ng/mL in the PCOS group.

Conclusion: The mean oxytocin level in the PCOS infertile women is lower than non-PCOS women. Oxytocin shows a significant reverse correlation with AMH and HOMA-IR.

Keywords: Polycystic Ovary Syndrome, Oxytocin, Anti Mullerian Hormone, Oral Glucose Tolerance Test

P-134: Comparison of Intravenous Immunoglobulins Injection in Infertility Patients with A History of Treatment Failure

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Background: Intravenous Immunoglobulins (IVIG) are widely used off label in the treatment of early reproductive failure. As IVIG is expensive, and may have side-effects, evidence of efficacy is needed. previous results have suggested that the pre-conception treatment of primary recurrent abortion patients might be effective, but the data set has been too small for adequate statistical power. More recently it has been suggested that IVIG may improve the success rate of *in vitro* fertilization and embryo transfer (ET) in patients with prior IVF failures, but clinical trials have given conflicting results that need explanation. systematic reviews generating reviews generating inconclusive result have focused on methodological rigor to the exclusion of biological plausibility.

Materials and Methods: From Jan 2016 to Feb 2017, A retrospective analysis was conducted of 30 consecutive patients attending infertility treatment center, who were the cases of recurrent abortion or recurrent failures of treatment. 15 patients randomly assigned to receive either the protocol with heparin (5000 U sq bid) IVIG (20g). Then the outcomes including laboratory pregnancy rate was compared across the groups.

Results: In this study, has been significant difference results was found ($P < 0.05$) by Chi-Square, Fisher's Exact, McNemar, Lambda, Goodman and Kruskal, Uncertainty Coefficient, Phi, Cramer's V, Mantel-Haenszel, Cochran's, and Kendall's Tau-b tests.

Conclusion: IVF outcome is significant improved when heparin and IVIG are administered women with repeat IVF failures.

Keywords: IVIG, Heparin, Recurrent Abortion

P-135: Mir-Pharmacogenomics Predictor Biomarkers of Metformin Effectiveness in PCOS

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Background: Polycystic ovary syndrome (PCOS) is a polygenic and complex disorder of reproduction system. It is one of the major causes of female subfertility. Polymorphisms of the candidate genes selected through pharmacogenomics can affect the variable drug response among PCOS patients. These genes are regulated by microRNAs (miRNAs). MiRNAs can be down-regulated by drugs or may affect drug metabolism and thus can

modify drug response; therefore they can be used as MiR-Pharmacogenomic biomarkers. MiR-Pharmacogenomic biomarkers can predict the effectiveness of metformin outcome in PCOS, based on the genetic susceptibility of individuals. Analyzing the Relationships between the microRNAs, target genes and drugs for prognostic drug response biomarker identification is available by bioinformatics tools. The main objective of this paper is *in silico* miR-Pharmacogenomics biomarker discovery of metformin outcome evaluation in PCOS based on bioinformatics tools.

Materials and Methods: PubMed articles published up to 2018 were searched by 'Polycystic ovarian syndrome', 'PCOS related genes', 'drug response in PCOS', 'metformin and PCOS', 'genetic variation in PCOS and metformin outcome' keywords. Afterward, *In silico* analysis by PCOSKB, Pharmaco-MiR, mTD, miRNA, miRmine and miRandola bioinformatics Tools was implemented.

Results: We used "PCOSKB" tool to obtain PCOS susceptible genes. High-value genes including AKR1C3, AMH, CGA, CYP11A1, HSD11B1, NR3C1, PI3, PPARG, SRD5A2, SST and TNF were selected. "Pharmaco-miR" and "mTD" tools were applied to predict microRNAs based on the relationships of mentioned genes and metformin effectiveness. Remarkable miRNAs were miR-181, miR-19, miR-125, miR-98, miR-539, miR-454, miR-138, miR-27, miR-130, miR-301, miR-320, miR-30, miR-124 and miR-183. Next evaluation of these Pharmacogenomics-miRNAs followed by miRNA and miRmine tools. In addition, the prognostic evaluation of achieved Pharmacogenomics-miRNAs as circulating biomarkers was analyzed with miRandola tool. Further verifications indicated that miR-128, miR-320 and miR-98 can be evaluated as efficient prognostic biomarkers of metformin effectiveness in PCOS individual.

Conclusion: Involvement of microRNAs in drug response provided the capacity of identification of novel prognostic and therapeutic biomarkers. The highly complex disease, PCOS, has a long-term complication with no exclusive (specific) treatment. The satisfactory results of identifying miR-Pharmacogenomics biomarkers in other complex diseases have provided hopes for the future pharmacotherapy development in PCOS. miRNA biomarkers profiling as personalized therapy can predict metformin dosage and efficacy responses based on genetic variation in PCOS patient. However, Further Experimental studies are necessary to investigate the usefulness of identified biomarkers.

Keywords: MiR-Pharmacogenomics, PCOS, Biomarker, Bioinformatics

P-136: The Effect of Vitamin E in Treatment of Infertile PCOS Patients

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Background: Vitamin E has multiple functions in humans and animals and its antioxidant effect was concluded in cancer therapy, high-risk pregnancy, and male infertility. several investigators have demonstrated the benefits of dietary supplementation

with vitamin E on fertility in different animal species. The aim of this study is to assess the effect of sufficient and insufficient levels of vitamin E in the treatment of infertile PCOS women.

Materials and Methods: In this clinical trial, 144 PCOS infertile patients referred to Dr. Rasekh clinic, Jarom, Iran that randomly divided into two groups (groups with sufficient and insufficient levels). Each of these two groups was randomly divided into case and control groups (36 participants in each group). Usual drug regimen of PCOS started for all groups (Metformin and dydrogesterone). Case groups received vitamin E supplementation as an add-on their treatment. Data collection performed via questionnaires by midwives and statistical analysis by SPSS 21.

Results: There was a significant relationship between follicular size and use of vitamin E ($P<0.05$). Increased endometrial thickness and reduced BMI detected in using vitamin E group. The overall pregnancy rate was twenty women (66.7%) which related to the using vitamin E groups.

Conclusion: Vitamin E has a positive effect on the treatment of PCOS patients. Although the response to the treatment is better in patients with insufficient vitamin E levels, prescription of this vitamin in patients with sufficient vitamin E level is also effective. According to rare side effects related to this vitamin, its low cost and low toxicity, we recommend adding the daily use of vitamin E to drug regimen of infertile PCOS patients.

Keywords: Infertility, Vitamin E, Pregnancy Rate

P-137: Compare the Effects of Herbal with Medical Drug in PCOS Infertile Woman

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Background: Polycystic ovary syndrome (PCOS), one of the most common causes of infertility due to anovulation, affects 4-7% of reproductive women. The purpose of this study is to compare the effect of nigella sativa + black pepper with letrozole + tamoxifen as a treatment of infertile polycystic ovary syndrome women.

Materials and Methods: This comparative clinical trial was done on 90 infertile PCOS women referred to Dr.rasekh clinic with aged 18-42 years. Patients were randomly allocated to either case or control group. The control group prescribed letrozole + tamoxifen and case group nigella sativa plus black pepper from third to eighth day of menstrual cycle. Transvaginal ultrasound parameters including ovarian follicular size, numbers and endometrial thickness were measured during treatment and based of this parameters continue these regimens and prescribed trigger drug.

Results: Pregnancy rate was higher in the group using nigella sativa plus black pepper and there was a significant relationship between two groups ($P<0.05$). Also, there was a significant relationship in endometrial thickness and dominant follicle size between the two groups ($P<0.05$). No significant correlation was found between two groups in the incidence of OHSS ($P>0.05$)

Conclusion: Because of significant effects of nigella sativa plus black pepper regimen on increase of endometrial thickness and size of dominant follicle, and eventually increase of pregnancy

rates. Therefore, we recommend, this low costs, low side effect regimen in treatment of PCOS patients.

Keywords: PCOS, Letrozole, Tamoxifen, Black Pepper, Nigella Sativa

P-138: Betaine Reduce The Gestational Diabetes Complication in Offsprings

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Background: Gestational diabetes mellitus (GDM) seriously impairs the health of maternity and infant, and it is also closely related to adverse pregnancy outcome. GDM affecting between 5-18% of all pregnancies worldwide. Mother in the period of high incidence season of diabetes also pose a significant risk on the growth and development of the fetus. The present study is aimed at investigating the antidiabetic effect of betaine and to analyse the reproductive outcome and fetal outcome in pregnant diabetic rats.

Materials and Methods: A total of 60 female Sprague-Dawley (SD) rats and 30 male SD rats weighing 180-200 g were selected. The animals were kept for two weeks as an acclimatization period prior to the start of the experiment and received normal basal diet and tap water ad libitum in a constant environment (room temperature $25 \pm 2^\circ\text{C}$, room humidity $55 \pm 5\%$) with a 12 h light and 12 h dark cycle. Vaginal smears were collected daily for estrous cycle determination, and then the estrous rats were paired with male rats without diabetes mellitus according to the rate 2:1 for conception. We checked female rats for vaginal plaque every morning for two days and those with vaginal plaque considered as a dam at day 0 of gestation. 32 dams divided into four equal groups: GDM, GDM+Betaine, Betaine and Control. The first and second group injected intraperitoneal Streptozocin (65 mg/kg) dissolved in 0.1M citrate buffer at day 0 of gestation and the third and fourth groups only treated by 0.1M citrate buffer. the animals in GDM+Betaine and Betaine groups treated with betaine (1.5% w/w) from day 5 to 20 of gestation. After delivery, we measured litter size, fetal weight and CR length of the pups in all groups. Blood samples were collected from all dams after delivery for HbA1c assessment.

Results: Litter size of GDM+Betaine group was significantly higher when compared to GDM group ($P<0.001$) and there was no significant difference between GDM+Betaine, Betaine and control group. Fetal weight in GDM group was significantly lower when compared to other groups ($P<0.05$). Crown-rump length of GDM+Betaine group was significantly increased compared to GDM group ($P<0.05$) and there was no significant difference between GDM+Betaine, Betaine and Control group. Glycated haemoglobin percentage (HbA1c%) as a diabetic index significantly increased in GDM group compared to all other groups ($P<0.05$). There was no significant difference for HbA1c% Values between GDM+Betaine, Betaine and Control group.

Conclusion: Our data suggest that betaine as a natural substance possesses protective effects against gestational diabetes mellitus deleterious role in fertility and it may be a good candidate to decline diabetes complication in women.

Keywords: Gestational Diabetes, Betaine, Offsprings, Rat

P-139: Effect of N-Acetyl cysteine on Expression of MTHFR Gene during Implantation Window in Women with Recurrent Implantation Failure: A Double-Blinded Randomized Placebo-Controlled Trial, Phase II

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Background: Despite significant developments in assisted reproductive technology (ART) that have overcome many underlying causes of infertility, pregnancy success rates remain relatively low, mainly due to implantation failure. Embryo quality and endometrial receptivity are two significant factors that believed to be the key points in implantation. The endometrium is receptive to blastocyst implantation during a spatially and temporally restricted window, called 'the implantation window. Folate metabolism is reported to be an essential regulator of early development and pregnancy. Methylene tetrahydrofolate reductase (MTHFR) plays a critical role in folate metabolism and in the homeostasis of homocysteine. Homocysteine is associated with oxidative stress through the production of reactive oxygen species (ROS) and nitric oxide regulation. N-acetyl cysteine (NAC) stimulates glutathione biosynthesis, promotes detoxification, and act directly as a scavenger of free radicals, therefore, it has been greatly applied as an antioxidant recently. The aim of this study is the assessment of NAC in implantation by monitoring the mRNA expression level of MTHFR gene.

Materials and Methods: A single center, double-blinded, placebo-controlled, randomized trial was performed over one year with 40 women (age: 22-40 years) with at least two RIF (Recurrent implantation failure) history who were undergoing *in vitro* fertilization (IVF) cycle. Forty infertile women with a diagnosis of RIF referred to Royan Institute were included in this study and randomly divided into intervention and control groups. At First, they received 1200 mg of effervescent tablets of NAC or the placebo for 6 weeks. The endometrial biopsy was taken from patients by catheter biopsy (Pipelle) on 19-21th day in the cycle prior to IVF transfer procedure. Total RNA-extraction and cDNA synthesis were performed on samples. Real-Time PCR was conducted to evaluate expression of MTHFR gene.

Results: The mean \pm SE of the fold change of MTHFR expression was 2.7 ± 0.4 in the drug group and was 2.4 ± 0.44 in the placebo group. No statistically significant difference were detected between two analyzed groups.

Conclusion: Related to our result, it seems N-acetyl cysteine effects on other mechanisms in implantation that needs more studies with larger population.

Keywords: Implantation, Recurrent Implantation Failure, N-

Acetyl Cysteine, MTHFR Gene

P-140: Effects of L-Carnitine on *In Vitro* Maturation and Developmental Potential of Oocytes Obtained from Transplanted Mouse Ovarian Tissues

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Background: Ovarian tissue transplantation is an emerging technology for fertility preservation. In addition, *in vitro* maturation of oocytes retrieved from grafted ovaries may overcome the fertility defects in some cases. The objective of this study was to evaluate the potential of using L-carnitine (LC) as an antioxidant to improve developmental potential of oocytes obtained from grafted ovarian tissues.

Materials and Methods: NMRI mice were divided into four groups: control (non-grafted), transplant (autograft without treatment), saline group (autograft +saline), LC group (autograft + LC). 6- weeks- old mice were ovariectomized and left ovaries were transplanted into the back muscle tissue. LC (150 mg/Kg) was injected intraperitoneally one day before surgical operation and repeated until one week after grafting. 3 weeks later, ovarian grafts were recovered and oocytes were harvested for *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* development (IVD).

Results: Our results indicated that the number of retrieved immature oocytes as well as successful IVM, IVF and IVD in transplanted groups was significantly lower than control group. ($P < 0.05$). All transplanted ovaries contained some oocytes that survived following IVM, IVF and IVD, and no significant difference was seen between grafted groups.

Conclusion: Our study demonstrates that L-carnitine alone did not show any negative effect on further development of oocytes. It seems that usage of LC in combination with a scaffold could improve autotransplantation results and more studies are needed in this area.

Keywords: Transplantation, L-carnitine, *In Vitro* Maturation

P-141: Hormonal and Metabolically Effect of Galega Officinalis in Adult Rats with PCOS

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Background: Polycystic ovary syndrome (PCOS) is one of the most incident reproductive endocrine illnesses in women, with an occurrence ranging from 5 to 21% in females of generative age. Aim of this study was evaluated the protective effect of *G. officinalis* on hormonal and metabolic parameters in adult rats with PCOS

Materials and Methods: Wistar female rats were allocated into sham (n=7) and experimental groups (n= 24) were induced PCO by single injection of estradiol valerate (16 mg/kg/IM), experimental groups were subdivided into 3 groups: G1, PCOS control group; G2, treated by G. Officinalis extract 200 mg/kg/orally/daily; G3, treated with hydroalcoholic extract of G. Officinalis 400 mg/kg/orally/daily. After the treatment period (2 weeks), all of the rats were anesthetized with ketamine and xylazine (50 and 10 mg/kg) then their blood samples were drawn and their serum samples were used for testing of glucose, insulin, Aromatase and hormones such as LH, FSH, Testosterone, and estrogen.

Results: The serum level of FBS, insulin, Aromatase, LH, FSH, Testosterone and estrogen was significantly increased in PCOS group as compared with control, and also in the treated groups that received 200 and 400 mg/kg of G. officinalis was significantly decreased when compared with PCOS group.

Conclusion: Results of the present research have been shown that extract of Galega officinalis has a significant effect on the level of the LH/FSH, testosterone, estradiol, aromatase, FBS, and insulin compared with PCOS group, which is maybe due to the presence of antioxidant and insulin-like agents, such as Bygvanydyn, resin, glycoside, and saponin.

Keywords: Galegaofficinalis, Hormone, Metabolic, PCOS, Rat

P-142: Effect of Boric Acid on Testes Development in Offspring from Induced Diabetic Pregnant Mice

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Background: Gestational Diabetes Mellitus (GDM) is one of the fast-growing clinical complications in the world. Gestational Diabetes Mellitus occurs when this placental hormones-induced insulin tolerance overcomes β -cells hyperplasia in pancreas. Studies showed that receive Boron in diet, change the pattern of insulin metabolism. We investigation the effect of Boric acid on testes development in offspring of maternal alloxan-induced diabetes mellitus.

Materials and Methods: For this aim, pregnant mice were divided into four groups: Control group received normal saline. Diabetic group that received 200 mg/kg alloxan intraperitoneally single dose at 3th days of pregnancy. Boric acid group that received Boric acid 10 mg/kg single dose on 6th, 8th and 10th days of pregnancy. Diabetic and Boric acid Group which received alloxan on 3th day of pregnancy to induce maternal Diabetes mellitus and then received Boric acid 10 mg/kg at 6th, 8th and 10th days of pregnancy. 60 days after birth, male mice were sacrificed, testes and caudal epididym removed for histological and sperm parameters analysis.

Results: Our results showed that Boric acid reduced the level of blood glucose in mothers compared to the Diabetic mother, also Diabetes reduced significantly the testes diameter, seminiferous tubules diameter and epithelium thickness and increased the lumen diameter compared to the control group while Boric acid improved all of these parameters, and sperm parameters showed that Diabetes significantly decreased sperm count, viability, motility and increased significantly sperm abnormality compared to the control group but Boric acid betterment all parameters.

Conclusion: So Boric acid can be a good choice for reduce the

advers effect of GDM on reproductive system specially spermatogenesis process

Keywords: Gestational Diabetes Mellitus, Boric Acid, Testis, Sperm

P-143: No Relationship between Serum Level of Progesterone on The Day of Transfer and Pregnancy Outcomes

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Background: Progesterone is an endogenous hormone secreted by ovaries that prepares uterine endometrium for implantation. The aim of this study was evaluating the relation of serum progesterone level on the day of transferring frozen embryo with pregnancy outcome.

Materials and Methods: 161 women were chosen for frozen embryo transfer. From the second day of their cycle, they were treated with 2 mg per 8 hours estradiol. From the 13th day, 5 mg inter muscular (IM) progesterone was injected every 12 hours to increase endometrium thickness above 8 mm. 4 days after progesterone administration, embryo transfer was performed and progesterone level was measured. Pregnancy outcome was also evaluated.

Results: The average progesterone level in 25 women with positive beta HCG was 26.26 ± 8.58 on 1st day, 26.63 ± 8.22 on 17th day, and 24.3 ± 11.48 on the 18th day of transfer. In 136 non pregnant women it was 25.61 ± 16.01 on 1st day, 29.03 ± 14.45 on 17th day, and 15.42 ± 2.77 on 18th day.

Conclusion: There was no relationship between serum level of progesterone on the day of transfer and pregnancy outcomes

Keywords: Frozen Embryo Transfer, Progesterone, Pregnancy

The Study of Relation Between CGG Repeats in FMR1 Gene and Molecular Genotype of Ovary in PCO Women with TP-pcr Technique

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Fragile X syndrome is recognized as a retardation that is dependent on chromosome X. In women this syndrome is accompanied with different degrees of mental retardation. The FMR1 gene was identified as the gene responsible for fragile X syndrome in 1991 and it plays a key role in ovaries function regardless of nervous effects. FMR1 mutation is the result of an increase in the number of CGG codon repetition. Polycystic ovarian syndrome (pco) is one of the most common endocrinology disorders in women, and the most common cause of infertility due to an absence in ovulation. In numerous studies conduct in countries other than Iran in the past, a close connection between an increase in CGG repetition in FMR1 gene and ovaries disorder was observed. Due to the importance of this disorder and the difference between gene pools in Iran and other countries, the aim of this study is to analyze the relation

between the number of triple CGG repetitions in FMR1 gene and poly cystic ovarian syndrome in women who visited Yas hospital with this syndrome between the years 1395-1396. Thus in order to conduct this study, we randomly selected 50 patients, extracted their DNA via their blood and determined the repetition number of CGG, using TP-PCR method. In this technique we used Forward primer that was marked with FAM (a fluorescent material). This technique determines whether or not the desired band exist, as well as the exact number of triple repetitions. In conclusion, after analyzing the obtained DNA sequences, only 7 patients with higher than normal CGG repetition were found and this limited number can not prove a reliable and solid connection between CGG repetition numbers and poly cystic ovarian syndrome in Iran's big population. Due to the vast variety in Iranian ethnicities, and the richness and difference in Iranian gene pool, it is possible to achieve better results with the help of professors and new techniques in the future.

Genetics

P-144: Anti-Apoptotic Effect of Royal Jelly Against Nicotine-Induced Testicular Injuries in Mice: Evidence of P53, Bcl-2 and Caspase-3 Expressions

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Background: Nicotine (NIC) as a toxic component adversely affects a male reproductive system. This study describes the effects of royal jelly (RJ) on testicular injury induced by NIC and elucidates the potential underlying mechanism at molecular level.

Materials and Methods: Thirty-six male BALB/c mice were randomly divided into six groups (n=6). Group 1 received normal saline, group 2 received 100 mg/kgBW/day RJ, groups 3 and 4 received NIC at doses of 0.50 and 1.00 mg/kgBW/day, respectively and groups 5 and 6 received NIC at doses of 0.50 and 1.00 mg/kg BW/day respectively plus RJ. Following 35 days, the expressions of P53, Bcl-2 and caspase-3 in testicular tissue were evaluated by RT-PCR.

Results: NIC treatment caused a significant (P<0.05) down-regulation of Bcl-2 expression and up-regulation of P53 and caspase-3 expressions versus control group. Nevertheless, RJ co-administration led to significant up-regulation of Bcl-2 expression and down-regulation of the expressions of P53 and caspase-3 versus NIC-exposed mice.

Conclusion: Our results confirmed that RJ effectively protects testis against NIC damages through its anti-apoptotic effects and mitochondria-dependent apoptotic pathway prevention.

Keywords: Nicotine, Royal Jelly, Apoptosis, Testis, Mice

P-145: HOX Gene Network Suggests Significant Association of Developmental Genes in Pathogenesis of Endometriosis

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Background: Endometriosis is a complex gynecologic disorder that affects as many as 10-15% of premenopausal women. The pathogenesis of endometriosis, as the presence of endometrium-like tissue outside the uterine cavity, is largely unknown. The purpose of this study is to identify causative genes in this disease with focus on HOX family genes and their regulating genes. HOX genes, encoding homeodomain transcription factors, are dynamically expressed in endometrium, where they are necessary for endometrial growth, differentiation, and implantation. Network theory allows for a holistic understanding of the role of genes in diseases.

Materials and Methods: PCR array data of 84 candidate genes used to construct co-expression network based on Pearson's correlation. The study performed on 15 ectopic, 15 eutopic and 15 normal tissues. Five tissues in each ectopic, eutopic and normal group were pooled as three biological repeats.

Results: Comparison of gene expression patterns between ectopic and eutopic with normal samples, revealed that the expression of 33 and 44 genes has significantly changed in eutopic and ectopic samples, respectively. Twenty two of genes were shared between two groups. The five genes of ISL2, HOXC12, LBX1, HOXC13 and EN1 exhibited the highest significant up-regulation (P<0.008) in eutopic vs. normal samples. ISL2 was not involved in any network. HOXC12 was a hub in a network. Some of its connections have been changed in eutopic samples. The node degree of LBX1 and EN1 was five and it involved in a network, which has not seen in the eutopic samples. HOXC13 was a hub in a network which has not seen in eutopic samples. The five genes MKX, DLX6, HOXB8, MSX2 and ARX exhibited the highest significance (P<0.0002) in the ectopic vs. normal samples. MKX and ARX were down-regulated and three other genes were up-regulated. MKX was a hub in a network which has not seen in ectopic samples. The node degree of DLX6 was four in normal samples whereas in the ectopic samples its connections have been changed. HOXB8 was not involved in any network in control samples while in the ectopic samples it belongs to a network and its degree was five. ARX and MSX2 belong to a network in normal samples but in the ectopic samples, MSX2 belongs to a large network and its degree was twelve while, ARX was not owned by any network in ectopic samples. ISL2 and EN1 play role in neurogenesis, MSX2 plays role in the apoptosis of neural crest. HOXC12, HOXC13, MKX, DLX6, HOXB8 and ARX play role in development and LBX1 plays role in the formation of muscle.

Conclusion: In the study, the most differentially expressed genes of patient vs. normal samples are effective genes in development. The study of co-expression networks between candidate genes showed that all of these genes, with the exception of HOXB8, have many interactions with the other genes studied, which are characteristic of developmental genes. These findings indicated correlation between developmental genes in formation of eutopic and ectopic endometrium cell.

Keywords: Endometriosis, HOX Gene Family, Network Theory

P-146: Sexing of Human Preimplantation Embryos without Embryos Biopsy through Rt-Pcr on Spent Culture Media

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Background: Preimplantation genetic diagnosis/screening (PGD/PGS) is a routine clinical procedure in many IVF clinics in the world that requires biopsy of embryonic cells or oocyte polar bodies. This is an invasive method to prevent the birth of children suffering from x-linked genetic disorders. Sex selection also is used for non-medical reasons like family balancing. The gender of the preimplantation embryo is important in some research. SRY gene expression begins in male embryos following genome activation in preimplantation embryos. The aim of this study was noninvasive sexing of preimplantation embryos using reverse transcriptase-polymerase chain reaction (RT-PCR) based on the presence of SRY RNA in the spent culture medium as a biomarker in sexing of human preimplantation embryos

Materials and Methods: In this double-blind study, two groups were evaluated, the first group had history of sexing by Fluorescent in Situ Hybridization (FISH) method through blastomere biopsy on the third day. These human embryos were cultured individually after biopsy. We received the spent media of the embryos on the fifth day. In the second group, embryos of ART candidates were individually cultivated on the third day until development to blastocyst stage; this group of embryos was not biopsied but the blastocysts were fixed for sexing by FISH. Each group was divided to 2 subgroups; in the first subgroup we extracted the RNA from the spent culture medium of each embryo. Following RNA extraction, the total RNA was immediately reverse transcribed to cDNA. In the second subgroup, PCR was performed directly on the culture medium. PCR was performed for SRY and GAPDH genes. The SRY positive embryos were considered as male embryos and those GAPDH positive and SRY negative were considered as females. Finally, the results of sexing based on culture media was compared with the results of sexing based on FISH.

Results: For the first group, 12 samples were evaluated. After comparing the results, we correctly diagnosed all 12 samples. For this group, the ability for correctly diagnosis of male samples was 100% (95% confidence interval: 67-100%) and the ability for correctly diagnosis of female samples was 100% (95% confidence interval: 30-100%) and the test accuracy was 100% (confidence interval 95%: 74-100%). In the second group, we were able to correctly diagnose 12 of the 14 samples; both embryos with a false diagnosis were of direct PCR. For the second group, the ability of correctly diagnosis of male samples was 100% (95% confidence interval: 40-100%) and the ability to correctly diagnosis of female sample was 80% (95% confidence interval: 45-98%) and the test accuracy was 86% (95% confidence interval: 58-99%).

Conclusion: Sexing of preimplantation embryos using RT-PCR technique on the spent culture medium without embryo biopsy seems to be a reliable tool for non-invasive preimplantation

sexing. However, direct PCR-based diagnosis might be lead to false results; this is probably due to DNA contamination.

Keywords: Preimplantation Genetic Diagnosis, SRY Gene, Embryo, Culture Medium

P-147: Expression of Interleukin (6, 8) and STATE3 Genes in Ectopic and Eutopic Endometrial Tissues

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Background: Endometriosis affects about 2 to 10% of women in fertile age, it may cause pain and infertility. The disease may spread into the abdominal cavity and even result in ileus. Deep endometriosis are located under the peritoneal surface, typically behind the uterus and in the region of uterosacral ligaments. Interleukin 6 (IL-6) is an inflammatory survival cytokine known to induce prolonged activation of STAT3 via association with the IL-6 receptor, activation of STAT3 signaling plays an important role in the pathogenesis of endometriosis. IL-8 takes part in all processes during the development of the disease: adhesion, invasion, and implantation of ectopic tissue. Additionally, the chemokine plays a role in growth and maintenance of ectopic endometrial tissue directly affecting endometrial cell proliferation. IL-8 might also protect ectopic cells against death by apoptosis. The purpose of this study was to determine the expression of IL6, IL8 and STAT3 in endometriosis.

Materials and Methods: RNA was extracted and reverse transcription was performed. Gene expression of IL6, IL8 and STAT3 was determined by RT-PCR.

Results: During this study, changes in the expression of this gene were observed in the ectopic tissue of patients with and without endometriosis.

Conclusion: Since IL6, IL8, STATE3 are the main factors in inflammation, differences in the expression of these genes might have an association with susceptibility to endometriosis. In RT-PCR based analyses of gene expression level in eutopic and ectopic endometrium, we strongly recommended that a differences of IL6, IL8, STATE3, genes are to be used.

Keywords: IL6, IL8, STAT3, Endometriosis

P-148: The Effect of Maternal Age on Gene and Protein Expression of YAP

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Background: The major events occurring during pre-implantation development is the separation of the two cell lines. The Hippo signaling pathway can play an essential role in lineage specification. If the pathway of Hippo is inactive, the YAP will enter the nucleus and cells are differentiated into the ICM and,

if active, YAP phosphorylated and remains in the cytoplasm and cells are differentiated into TE. There may be several variables that can have effect on gene expression, one of these variables investigated in this research is mother's age. This study compares YAP gene and protein expression between blastocyst embryos of maternal aging 20s and over 37 years.

Materials and Methods: Human, day 2 or 3, embryos with maternal age of 20s (as Young group) and over 37 (as Older group) are cultured up to blastocyst stage. Then, quantitative expression of YAP gene and protein in both groups was evaluated using qRT-PCR and immunostaining methods respectively.

Results: There was no significant difference in the expression of YAP gene in blastocyst stage between Young and Older groups. The results of immunostaining showed that the localization of protein expression of YAP and P-YAP in mothers over the age of 37 years was not suitable.

Conclusion: The results of this study showed that, as the age rises, the expression pattern of Hippo signaling components (YAP, P-YAP) change in human cell lineage of blastocyst.

Keywords: Maternal Age, Hippo Signaling Pathway, YAP and P-YAP

P-149: Improved Blastocyst Attachment through Integrin $\beta 3$ Up-Regulation in Mice

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Background: Despite the encouraging advancement in fertility technology, success rate of ongoing pregnancy is relatively low which is mainly related to implantation failure. Recent studies have suggested the probable role of miRNAs in regulation of numerous critical genes during implantation. Integrins, a group of adhesion molecules, are post-transcriptionally regulated by miRNAs. Integrin receptors are involved in blastocyst apposition and attachment. Among various integrin subunits, mir-Let-7a targets Integrin $\beta 3$ (Itg $\beta 3$). Hence, this study aimed to evaluate the effect of Let-7a down-regulation on the implantation rate through regulation of integrin $\beta 3$ expression level in blastocysts derived from microinjection in mice.

Materials and Methods: Towards this goal, anti-mir-Let-7a vector was transmitted to embryos using two different methods. In the first method, the vector was injected into *in vivo* (MII) and *in vitro* (GV-MII) matured oocytes using microinjection. In the second method, the vector was electroporated into eight cells embryos obtained from Intracytoplasmic sperm injection (ICSI). The expression levels of Let-7a, and Itg $\beta 3$ were evaluated using qRT-PCR, immunocytochemistry and western blot. Next, we isolated mouse endometrial cells to assess the embryo attachment. Blastocyst attachment on the endometrial cells was monitored via microscopic technique.

Results: QRT-PCR analysis showed no considerable change in Let-7a expression level after anti-mir-Let-7a transmission by either microinjection or electroporation in comparison with the control group. While Itg $\beta 3$ was significantly up-regulated following anti-mir-Let-7a transmission ($P < 0.05$). Trophoblast cells attachment and migration to endometrial cells was dramati-

cally increased by approximately 25% compared to the control group ($P < 0.05$).

Conclusion: It is concluded that Let-7a could mediate the embryo attachment by regulation of Itg $\beta 3$ expression level.

Keywords: Attachment, MicroRNA, Intracytoplasmic Sperm Injection, *In Vitro* Maturation, Electroporation

P-150: Effect of Vitrification on Expression of Apoptotic Genes in Mouse Metaphase II Oocytes following Cryotop Method

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Background: Vitrification is an effective approach for cryopreservation of human oocyte and embryos. The aim of this study was to investigate the vitrification effect on Bax and Bcl-2 expression level in mouse metaphase II (MII) oocytes.

Materials and Methods: The survival and fertilization rate of MII mouse oocytes was assessed following vitrification by cryotop. Oocytes were randomly selected and distributed amongst the five experimental groups (control, docetaxel, docetaxel + VS (Vitrification solution), docetaxel + Vitrification, and vitrification). Then, vitrification method effect on the expression of Bax and Bcl-2 genes were determined in vitrified-warmed oocytes by real-time RT-PCR. Each group was compared with the control. Data were analyzed with ANOVA using Graph Pad and SPSS version 21 software.

Results: There were significant differences between Bax gene expression level in the control group with the docetaxel and vitrification groups ($P < 0.05$). Bcl-2 gene expression level was significantly high in the vitrification group (vitrification + Docetaxel) and non-vitrified group (Docetaxel + VS) ($P < 0.01$).

Conclusion: This study indicated that the vitrification of mouse MII oocytes can increase Bax and Bcl-2 genes expression.

Keywords: Vitrification, Oocytes, Docetaxel, Bax, Bcl-2

P-151: Expression of Toll-Like Receptor 5, MyD88 and NF- κ B in Eutopic and Ectopic Endometrium Tissues of Women with Endometriosis

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Background: Endometriosis is a multifactorial disease that causes pelvic pain and infertility in women of reproductive age. Previous studies indicated that the growth of endometriosis can be regulated by the innate immune system in the pelvic environment. Different immune cells and dendritic cells express Toll-like receptors (TLRs) and respond in a specific way to pathogens. TLRs play a major role in endometrial defense against microorganism. Here, we examined role of TLR5, MyD88 and NF- κ B during all of the menstrual cycle.

Materials and Methods: The Expression of TLR5, MyD88 and NF- κ B mRNA in Eutopic and Ectopic Endometrial Tissue from Women with Endometriosis Compared to endometrial tissues of control group.

Results: The expression of TLR5 and MyD88 mRNA was dif-

ferent in both eutopic and ectopic endometrial tissues of women with endometriosis compared to control group.

Conclusion: Different expression of TLR5 and MyD88 in eutopic and ectopic endometrial tissues may be involved in the inflammatory pathogenesis of endometriosis.

Keywords: TLR5, MyD88, NF-kB, Endometriosis

P-152: miR-15a Expression Pattern in Spermatozoa of Patients with Varicocele Grade III

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Background: Varicocele is one of the leading causes of male infertility, which in some severe cases will lead to defect in the process of spermatogenesis, followed by changes in spermogram and infertility. Much controversy still exists regarding the diagnosis, management and pathophysiology of spermatogenesis alterations associated with varicocele. However, heat-induced changes in the testis following the occurrence of varicocele and the activity of the proteins involved in the heat shock pathway have been implicated in development of spermatogenesis failure. Evidences indicate that microRNAs (miRNAs) are engaged in regulation of heat shock proteins. We evaluated the expression patterns of miR-15a as a heat shock-related miRNA in the spermatozoa of men from three different groups; patients with varicocele grade III and abnormal spermogram, patients with varicocele grade III and normal spermogram and finally normal control men.

Materials and Methods: The samples as fresh ejaculate were collected from 15 men in each group during May 2016 till August 2017, at Royan infertility center. Semen was subjected to a density-gradient centrifugation (DGC) to collect spermatozoa for subsequent RNA extraction. Subjects were studied by quantitative real-time polymerase chain reaction (qRT-PCR) on RNA extracted from ejaculated sperm to analyze miR-15a expression throughout the three groups.

Results: The expression of miR-15a was significantly decreased in patients with varicocele grade III and abnormal spermogram compared to the cases with varicocele grade III and normal spermogram ($P=0.04$) and cases from control group ($P<0.001$). In addition, we detected a relative increase in miR-15a expression in patients with varicocele grade III and normal spermogram compared to control group ($P=0.423$).

Conclusion: Our results may suggest protective effects by miR-15a on the spermatogenesis process in a form of threshold. Pathological trends in varicocele are possibly accompanied by a relative reduction of such regulatory factors. Our results provide a valuable insight into the varicocele-related sperm impairment and may help to develop potential therapeutic targets and novel biomarkers for male infertility. It could be used as an important prerequisite to the development of diagnostic tests to predict varicolectomy outcomes in patients with varicocele and abnormal spermogram.

Keywords: Sperm, Varicocele, Heat Shock Pathway, microRNA

P-153: Evaluation of Sperm Chromatin/DNA Integrity, Morphology and Catsper Expression on Diabetic C57BL/6 mice

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Background: Diabetes is associated with reproductive impairment on the male reproductive system and causes complications such as decreased libido, fertility, spermatogenesis, sperm motility, and morphology. High level of blood sugar may affect sperm quality and reduces the potential for male fertility. Increased levels of sperm DNA damage is often associated with reduced count and motility or abnormal morphology.

Materials and Methods: This experimental study was performed in Mashhad University of Medical Sciences. 40 mice (C57BL/6) were divided randomly into 4 groups: 1. Control group, 2. Diabetic group, 3. Diabetic + Insulin group, and 4. Sham group. After 35 days, the right epididymis of all specimens was used for Real-Time PCR and left epididymis for evaluation sperm parameter using Aniline blue, Toluidine blue, Papanicolaou, and immunohistochemical study. Also, right testes were applied for immunohistochemical and tunnel study.

Results: Results of this study showed that chromatin integrity, morphology, and cation channels of sperm (Catsper) expression was significantly changed in diabetic mice in comparison to other groups ($P<0.05$) and treatment with insulin improved these parameters.

Conclusion: Our findings showed that the sperm parameters such as DNA integrity, morphology, and Catsper expression change in diabetic mice.

Keywords: Diabetes, Sperm, Chromatin, CATSPER, Mouse

P-154: Alterations of Histone H3 Trimethylated at Positions K4 and K27 in Zygote Genome Activation Stage of Mouse

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Background: Histone modifications have serious roles in regulating the expression of developmental genes during embryo development in mammals. Methylation of histones is one of the most important epigenetic modifications and has a critical role in both transcriptional repression and activation during embryonic development. Histone H3 lysine 4 trimethylation (H3K4me3) is an epigenetic marker associated with active transcription, and it has a close relationship with histone acetylation and deacetylation. Also, histone H3 lysine 27 trimethylation (H3K27me3) is involved in silencing of gene expression. On the other, first main developmental event that occurs following fertilization is Zygotic gene activation. This episode is responsible for dramatic reprogramming of gene expression that occurs during the 2-cell stage of the mouse. Therefore, the

aim of this study was to investigate the distribution patterned of H3K4me3 and H3K27me3 in 2-cell stage of mouse embryo using immunofluorescence staining.

Materials and Methods: Two cell embryos fixed 24 hours after insemination and stained with specific antibody for modification in H3K27me3 and H3K4me3. The fluorescence images investigated and relative intensity calculated using the Image-J. Data analyzed by two independent sample t-tests. Differences were considered as significant at $P < 0.05$.

Results: The results indicated H3K4me3 and H3K27me3 signals were 40.29 ± 6.03 , 25.28 ± 2.02 respectively. A sharp increase in the intensity of H3K4me3 observed that was significantly higher than H3K27me3 in 2 cells.

Conclusion: The changes in gene expression occur in ZGA so the levels of H3K4me3, which is involved in gene regulation; show dramatically increased likely has amplified gene expression in the major wave of zygotic genome activation in the two-cell mouse embryo.

Keywords: H3K4me3, Mouse, H3K27me3, Zygote Genome Activation

P-155: Evaluation of Cluster in Gene Expression in Infertile Man

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Background: Infertility refers to the inability of couples to achieve successful pregnancy after a year of sexual intercourse without prevention and occurs in both males and females for various reasons. About half of the infertility causes is due to male factors such as azoospermia. Azoospermia is a male disorder observed in 1% of the general population and in 10-15% of infertile men that is defined as the absence of spermatozoa in the ejaculate. Azoospermia is divided into two types: non-obstructive azoospermia and obstructive azoospermia. Approximately 60% of these cases are due to non-obstructive azoospermia, a condition in which men have impaired spermatogenesis, the process of sperm production. Non-obstructive azoospermia men require testicular sperm extraction (TESE) for sperm retrieval with assisted reproduction techniques to allow fertility. Spermatogenesis is an extraordinary complex process which participation of several cell types, hormones, paracrine factors and genes are required for the differentiation of spermatogonia into spermatozoa. Therefore, investigating the factors involved in spermatogenesis, including genes, is one of the important aspects in understanding the mechanism of infertility in men. Deficiency in the expression levels of such genes may lead to spermatogenesis failure. Clusterin as a candidate gene has been shown to play important roles in several pathophysiological processes, including tissue remodelling, lipid transport, reproduction, complement regulation, apoptotic cell death and sperm maturation. To end this, in this project we aimed to understand the role of Clusterin gene in spermatogenesis process, the expression of this gene was investigated in the testis tissue in non-obstructive azoospermia patients.

Materials and Methods: The study population included 42 infertile Non-obstructive azoospermia men referred to Royan

institute. Based on the results of their testicular biopsy, patients were categorized into two groups: TESE+ (positive sperm retrieval) and TESE- (negative sperm retrieval). The testicular tissue samples were obtained from the surgery for molecular analysis. The genomic RNA was then extracted by Trizol and converted to cDNA. The gene expression was investigated by Real-time PCR technique. The obtained data were analyzed using SPSS the18 software. The $P \text{value} \leq 0.05$ was considered significant.

Results: The present study showed that based on the Real-time PCR results, the expression level of clusterin gene in TESE+ group was significantly higher than TESE- group ($P = 0.035$).

Conclusion: According to the results of this limited study, the Clusterin gene expression may have a role in spermatogenesis as its expression was significantly low in patients with no sperm even after TESE surgery. By evaluating expression of Clusterin gene associated with its protein expression in the semen plasma in a larger population of non-obstructive azoospermia patients, more receivable information would be gained.

Keywords: Male Infertility, Non-Obstructive Azoospermia, Sperm Extraction, Clusterin Gene Expression

P-156: Alteration in Expression of Genes Involved in The Initial Steps of Steroidogenesis in Mice Abdominal Adipose Tissue by Dietary Saturated and Trans Fatty Acids

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Background: Adipose tissue (AT) constitutes an important site for steroid hormone metabolism. It seems that eating habits such as oils with higher content of saturated or trans fatty acids (FA) are the main causes of subfertility. However, to our knowledge, the question of whether consumption these FA affects steroidogenesis in AT has not been addressed. The current study was designed to determine whether dietary saturated or trans-FA influences the expression of two rate-limiting factors of steroidogenesis, i.e., steroidogenic acute regulatory protein (Star) and cytochrome p450 (CYP11A1) in male mice AT.

Materials and Methods: Mature male mice (8 weeks) were divided into the fat diet (FD) ($n=15$) and control ($n=14$) groups. FD group fed tallow for 2 months and abdominal AT samples were collected and performed quantitative RT-PCR.

Results: The levels of Star transcripts in FD group were significantly lower compared to the control group. The levels of CYP11A1 in FD group significant higher compared to the C group ($P < 0.05$).

Conclusion: A significant increase in the mRNA abundance of CYP11A1 indicates that AT expresses the CYP11A1 in the pathway from cholesterol to active steroid hormones and de novo steroid synthesis in AT. The present results demonstrate

the stimulatory effects of saturated or trans FA on the initial steps of steroidogenesis in AT as well as hormonal imbalance in male which warrants further studies.

Keywords: Star, CYP11A1, Adipose Tissue, Steroidogenic

P-157: High Resolution Genotyping of AZFc Partial Deletion to Detect Exact Gene Losses and Determine The Potential Phenotype of Spermatogenic Impairments

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Background: Variable defined phenotypes, from azoospermia to oligospermia, for partial deletion of AZFc are known as deletion of different copies or significant gene loss. Remove two copies of the DAZ gene, one copy of CDY1 and one copy of the BPY2 gene in the gr/gr deletion are indicative of the importance of this to determining the spermatogenic impairment. This study aimed to determine the best useful genetic plan to access partial deletion of AZFc.

Materials and Methods: From a total of 140 infertile men referred to the Dastgheib Hospital, Shiraz, Iran, 15 samples with AZFc microdeletion enrolled to genotype partial deletion. Sequence tagged sites (STS) in each palindromic structures of this region detected to genotyping patients for the presence or loss of each copy of genes.

Results: Screening markers SY1198 in the g1, g2 and g3 and sY1189 in the distance between r2 and b3 of AZFc can complete previous defined marker plan (presence of SY1191 and deletion of sY1291) to detect deletions of gr/gr of AZFc. SY1189, SY1291 and SY142 markers can employ to increase the accuracy of identifying the deletion of b2/b3. SY1313 is an informative marker to differentiate CDY1b gene from its aligned copy, CDY1a gene, which presence of sY1313 and absence of sY1190 is indicative of CDY1a gene deletion.

Conclusion: We determined ten STS markers; sY1189, sY1190, sY1201, sY1191, sY1291, sY1198, sY142, sY1313, sY254 and sY255 to high resolution genetic mapping and detect exact deletion of the region and gene copy loss in the AZFc region.

Keywords: Male Infertility, Azoospermia, Y Chromosome, AZFc, STS Marker

P-158: Evaluation of The Interleukin 10, Toll-Like Receptor 2, 4 and Heat-Shock Protein 70 Expression in Endometriosis Lesions Versus Normal Controls

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Background: Endometriosis is an inflammatory disease which affects females of reproductive age. The retrograde menstruation is the major theory of the pathogenesis of endometriosis which cause inflammation in peritoneal cavity and may lead to reproductive failure. Tissue damage and tissue invasion in endometriosis may result in releasing endogenous heat-shock proteins (HSPs) in the pelvic environment. HSP70 is intracel-

lular protein which be recognized by toll-like receptors and can express IL10, an anti-inflammatory cytokine with multiple effects in immunoregulation and inflammation. The aim of our study is to investigate the expression of HSP70, TLR2, 4, IL10 in menses, proliferative and secretory phases of menstrual cycle and their differences in eutopic and ectopic tissues of endometriosis.

Materials and Methods: To evaluate the expression of target genes, ectopic and eutopic tissues of endometriosis patients in different phases of menstruation was collected and reverse transcriptase polymerase chain reaction (RT-PCR) was performed on the prepared cDNA samples with the use of primers designed for TLR2, TLR4, HSP70, IL10.

Results: Data analyses indicate different expression of the genes in various phases of menstruation in eutopic and ectopic lesions.

Conclusion: This study has shown that alteration in the expression of TLR2, TLR4, HSP70, IL10 through TLR signaling pathway might have an important role in endometriosis symptoms, therefore evaluation of the expression of these genes in endometriosis patients is highly recommended.

Keywords: Endometriosis, HSP70, TLR2, TLR4, IL10

P-159: Aberrant Expression of Imprinted Gene Mest (Peg1) in Blastocyst-Stage Mouse Embryos following IVF and Embryo Culture

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Background: Genomic imprinting, a specialized epigenetic mechanism of transcriptional regulation, results in monoallelic expression of a group of mammalian genes. Disturbances in these asymmetric parental states can have intense consequences for growth and development such as Beckwith-Wiedemann syndrome (BWS) and Angelman syndrome. Preimplantation embryos are vulnerable to epigenetic modifications because extensive epigenetic remodeling takes place during early development. Thus in this study expression of Mest (Peg1), one of the most important imprinted genes, in blastocysts obtained from *in vitro* fertilization (IVF) was compared with fresh blastocysts in a mouse model.

Materials and Methods: In control group, fresh blastocysts were collected from uterine horns of superovulated mice 94 h post-HCG. For IVF group, *in vitro* produced zygotes were cultured in KSOMaa medium supplemented with 10% bovine serum albumin (BSA) under mineral oil for 96 h at 37 °C in a humidified atmosphere of 5 % CO₂ and 95 % air up to blastocysts stage. qRT-PCR was carried out to evaluate the frequency of the levels of Mest (Peg1) gene in the blastocysts.

Results: The result showed that quantitative level of Mest (Peg1) gene was significantly higher in blastocysts derived from IVF in comparison to fresh blastocysts (p<0.05).

Conclusion: In conclusion, IVF and preimplantation embryo cause disruption in Mest (Peg1) gene in blastocysts.

Keywords: IVF, Epigenetic, Genomic Imprinting, Mest, Blastocyst

P-160: Effect of Ovulation Stimulation on Epigenetic Pattern of Imprinted/Developmental Genes in NMRI Mice

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Background: Genomic imprinting is an epigenetic phenomenon that plays a critical role in normal development of embryo. It has been proven that deficient genomic imprinting can cause infertility. Changes that occur in the hormonal profile, reproductive system and gametes of an organism during assisted reproductive technology (ART) can affect epigenetic events. The aim of this study was to evaluate the genetic and epigenetic changes in a few body organs after ovarian stimulation. For this, the expression of three imprinting genes (H19, Igf2 and Cdkn1c), all of which have important roles in development of placenta and embryo.

Materials and Methods: The epigenetic profile of their regulatory region in brain, lung, heart, liver, kidney, ovary and placenta of 19-day-old female mice fetuses subjected to ovulation induction were evaluated by qRT-PCR and ChIP methods.

Results: Results showed altered gene expression in line with changes in epigenetic pattern of their promoters in the ovulation stimulation group vs. normal cycle. H19 gene decreased significantly in lung, heart, liver, placenta and ovary of ovulation stimulation group in comparison to control fetuses. However kidney showed increased levels of H19 in mentioned experimental group vs. control. Igf2 showed significantly higher levels of expression in brain and kidney and Cdkn1c showed significant increase in lung and significant decrease in placenta of ovarian stimulation group versus control fetuses.

Conclusion: Disrupted gene expression pattern and epigenetic profile of these imprinting genes may be the cause of low weight syndrome in embryos or incomplete growth of organs. Also some problems like respiratory distress syndrome in ART newborns or urogenital deficiencies in ART born children may be related to disruption of these genes expression in their lung and kidney, caused by ovarian stimulation.

Keywords: Epigenetic, Genomic Imprinting, Ovarian Stimulation

P-161: Assessment of c.474G>A Variation Effect on SEPT12 Expression Pattern and Annulus Status in Teratozoospermic Men

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Background: Septins belong to the family of cytoskeletal GTPase proteins usually mediate their biological processes through elongation into higher order structures including filaments and rings. These proteins are considered as fundamental constituents of human sperm tail annulus (an electron-dense ring structure demarcates the midpiece and the principal piece of sperm tail). SEPTIN12 is a testis-specific gene potentially involved in terminal differentiation of male germ cells. The SEPT12 is widely expressed at various subcellular regions of post-meiotic germ cells in humans and is located at the head and neck of spermatids and the annulus of mature spermatozoa. An exonic variant, c.474 G>A, located within GTP binding domain of SEPT12 induces a cryptic splice donor site results in creation of premature stop codon and translation of a truncated protein. In the current study we investigated the effect of c.474G>A variation on expression pattern of this protein and also annulus status in men with teratozoospermia.

Materials and Methods: In this study 30 infertile men with teratozoospermia and 30 normozoospermic men as control group were recruited. To investigate genetic variations, DNA was extracted from peripheral blood samples using salting out method and mutational analysis was performed by direct sequencing. Besides, we screened sperm smear preparations by immunofluorescence detection assay in individuals carrying c.474G>A variation.

Results: c.474G>A was detected in 8 patients with teratozoospermia and 4 normozoospermic men. In normozoospermic and teratozoospermic patients carrying c.474G>A heterozygously, annulus was detected but SEPT12 signals were likely weaker than control individuals. In addition to, loss of annulus, punctate expression pattern and a variable signal intensity of SEPT12 were clearly observable in some spermatozoa of these patients. Interestingly, analysis of SEPT12 immunoreactivity in normozoospermic and teratozoospermic cases with c.474G>A in homozygous state represented similarity to heterozygous individuals and most of the spermatozoa from these patients possessed annulus.

Conclusion: We concluded that, annulus detection and similarity in SEPT12 expression in teratozoospermic and normozoospermic individuals carrying c.474G>A in homozygous and heterozygous states could be arisen from two phenomenon. Firstly, the relative abundance of mutated SEPT12 may be too low to disrupt annulus integrity. Secondly, nonsense-mediated mRNA decay pathway eliminates aberrant mRNA transcripts that contain premature stop codons result in encoding incomplete polypeptides. So our data indicates that there would be no association between SEPT12 c.474G>A nucleotides variation and annulus status in teratozoospermic men with Iranian origin.

Keywords: c.474G>A, Teratozoospermia, SEPT12 Expression, Annulus

P-162: Comparison of Y Chromosome Azfc Partial Microdeletions in Azoospermic and Fertile Male Groups Referred to Royan Institute

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Background: Microdeletion of the azoospermia factor (AZF) region of Y chromosome is an important genetic cause of male infertility. AZFc partial deletions result from homologous recombination and may be classified in gr/gr, b1/b3 and b2/b3 groups. AZFc region contains eight gene families expressed mainly in the testes. Deletions of this region lead to reduced copy numbers of these genes were reported to be associated with diseases such as male infertility and testicular germ cell tumors. Several studies have reported that AZFc partial deletions affects the quality of sperm produced and may be a cause of germ cell loss/degeneration leading to azoospermia or oligozoospermia. We aimed to evaluate the types as well as frequencies of AZFc partial deletions among Iranian azoospermic and fertile men referred to Royan institute.

Materials and Methods: Multiplex PCR with seven sequence tagged site (STS) markers were used to determine the AZFc partial deletions in a total of 200 infertile men with non-obstructive azoospermia and 200 normozoospermic controls. The chi-square test will be used for statistical analysis ($P < 0.05$).

Results: The research is ongoing. Till now, a total of 120 men were evaluated where 11 (9.6%) detected with one of the AZFc partial deletions. Among normozoospermic men, 2 out of 66 (3%) and from azoospermic men 9 out of 54 (16.6%) had AZFc partial deletions. Statistical analysis shows a significant higher frequency for AZFc partial deletions in azoospermic men compared to controls (16.6 vs 3.0%) ($P = 0.022$).

Conclusion: In conclusion, based on our preliminary data, AZFc partial deletion may be considered as a risk factor for male infertility. However, the roll of AZFc partial deletion on spermatogenetic impairment remains controversial and further studies are needed to better clarify this phenomenon. The screening for AZFc partial deletions may help the clinicians in determining the cause of male infertility and decide a reasonable strategy for the patients. Since these deletions are transmitted to all of male offspring born through assisted reproduction, testing for AZFc partial deletions will allow the couples to make an informed choice regarding the perpetuation of male infertility in future generations.

Keywords: AZFc Partial Deletions, Y Chromosome Deletion, Azoospermia, GR/GR, Male Infertility

P-163: Rate of Heterozygosity of two Short Tandem Repeat Markers from Chromosome 18 in Iranian Population.

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Background: Preimplantation genetic screening (PGS) involves biopsy of a single cell from a cleavage stage embryo fertilized through intra-cytoplasmic sperm injection (ICSI). Majority of women seeking assisted reproductive techniques (ART) are women older than 35 years olds and PGS may help such couples to select embryos with normal chromosomal complement. PGS screens the embryos for numerical chromosomal abnormalities including trisomies 13, 18 and 21. Trisomy 18 (Edwards syndrome) is caused by an error in meiotic disjunction. Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) is a technique for detection of aneuploidies in prenatal stage and works based on heterozygosity of short tandem repeat (STR) markers for particular chromosomes. With the aim of setting up this method in preimplantation stage we evaluated the heterozygosity and polymorphic rate of D18S976 and D18S535 STR markers in Iranian population.

Materials and Methods: Genomic DNA was extracted from peripheral blood of a total of 50 healthy men and women. D18S976 and D18S535 STR markers were amplified using specific primers and multiplex PCR. The PCR products were run on acrylamide gel and the percent of heterozygosity of each marker was calculated separately.

Results: We observed homozygote genotype for D18S976 and D18S535 markers in 32 and 38% of the cases, respectively. Therefore, 68 and 62% of cases were heterozygote for D18S976 and D18S535 markers, in respect.

Conclusion: Previous studies have reported D18S976 and D18S535 as informative markers in diagnosis of trisomy 18 in other populations. According to our results, these markers seems to be relatively informative of trisomy 18 for Iranian population as well, although it is necessary to be evaluated in larger population.

Keywords: Preimplantation Genetic Screening, Short Tandem Repeats, Trisomy 18, D18S976, D18S535

P-164: Expression Profile of HOXB 6-13 Genes in Cumulus Cells of Women with Polycystic Ovaries

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Background: 10-15 percent of women in fertility age suffer from polycystic ovarian syndrome (PCOS). PCOS is a woman-related disease that is recognized by existence two of the three criteria: hyperandrogenism, oligo anovulatory and polycystic ovary, that 20% of normal female population may show Polycystic ovary disease (PCOD) in ultrasound definition, which have normal hormone concentration and menstrual cycle. Genetic disorder is one of the most important PCOS's factors affecting oocyte and it's around tissue such as cumulus cells during folliculogenesis. Matter of HOX genes as master genes in cell function and development is studied in many dis-

eases. In this research, the expression profile of HOXB6-13 genes in the cumulus cells of women with PCOS and PCOD were focused.

Materials and Methods: The study groups were included of 22 PCOS and 8 PCOD versus 10 normal women. After RNA extraction and cDNA synthesis, mRNA expression of the candidate genes was measured by real-time PCR.

Results: Data showed increased expression of HOXB6 ($P=0.002$), HOXB7 ($P=0.002$), HOXB8 ($P=0.02$), and HOXB13 ($P=0.001$) in cumulus cells of women with PCOS vs. control. However, there were no significant changes in mRNA levels of the aforementioned genes in PCOD group vs. control and nor PCOS.

Conclusion: This study give us an idea about a significant correlation between altered expression of HOXB members and PCOS disorder, and implies the dynamic role of these developmental genes in pathogenesis of polycystic ovary symptoms.

Keywords: HOXB Genes, PCOS, PCOD, Cumulus Cells

P-165: Interactions Between Cyclin D1, cdk-4, p21 and PCNA in Testicular Torsion and Reperfusion Model in Rats; Evidence for Germ Cells DNA Damage and Development

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Background: The current manuscript is provided to analyze the cross-link between cyclin D1, cdk-4, p21, PCNA and DNA damage during different period of reperfusion following experimental torsion in rats.

Materials and Methods: To follow-up current study, 30 mature male Wistar rats (NO=6 rats in each group) were used. Following 4 hours from torsion induction, the reperfusion was induced. Then, the animals were subdivided into; 4 hours torsion-induced (T1), (b) 1hour post-reperfusion (T2), 2 hours post-reperfusion (T3), 4 hours post reperfusion (T4) and 8 hours post-reperfusion (T5) groups. The seminiferous tubules differentiation (TDI) and spermiogenesis (SPI) indices were investigated and compared between groups. The cyclin D1, cdk-4, p21, PCNA expressions were analyzed using Reverse Transcriptase-PCR (RT-PCR). Moreover, the cyclin D1+, cdk-4+, p21+ and PCNA+ cell numbers per mm² of tissue were assessed using immunohistochemical staining. Finally, the testicular DNA fragmentation was analyzed using DNA ladder test.

Results: Observations demonstrated that, reperfusion (albeit after 8 hours) significantly ($P<0.05$) up-regulated the cyclin D1, cdk-4 and PCNA expressions versus T1 group. Moreover, the animals in T5 group exhibited diminished expression of p21 and represented diminished DNA fragmentation versus T1 group. No statistically significant ($P>0.05$) differences were revealed between T1, T2, T3 and T4 groups for all analyzed parameters.

Conclusion: In conclusion, minimum 8 hours, post reperfusion is needed to re-initiate necessary expressions of cyclin D1, cdk-4 and PCNA to restore cell cycling machinery and ameliorate torsion-induced DNA damage.

Keywords: Torsion, Reperfusion, DNA Damage

P-166: Evaluation of Crucial Cell Junction Genes in Endometriosis

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Background: Endometriosis is a condition in which the endometrium, the layer of tissue that normally covers the inside of the uterus, grows outside of it. Tight junctions are regulated in their molecular composition ultrastructure, and function by intracellular scaffolding proteins and the cytoskeleton; such regulation serves normal, physiologic adaptation but also occurs in numerous diseases. It has however, become increasingly apparent that the tight junction has a vital role in maintaining cell to cell integrity and that the loss of cohesion of the structure can lead to endometriosis. The aim of the study was to assess tight junction genes functions in the context of endometrial biology with the usage of RT-PCR.

Materials and Methods: This research evaluated the expression of cell junctions genes of desmoglein-1, E-cadherin and zona occludin-1 as reliable reference genes in eutopic and ectopic endometrial tissue specimens obtained during standard surgery of women of reproductive age.

Results: The candidate gene expression altered in groups in compared with control group.

Conclusion: We describe a genetic basis for endometriosis and provide strong evidence for the existence of the role of tight junction abnormality in endometriosis patients.

Keywords: Endometriosis, Cell Junction, E-Cadherin, Desmoglein-1, Zona Occludin-1

Effect of vitrification on expression of apoptotic genes in mouse Metaphase II (MII) oocytes following cryotop method

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Background: Oocyte cryopreservation is considered as an important component of human assisted reproductive technology (ART). Vitrification is a kind of cryopreservation that used for freezing of human oocyte and embryos. The aim of this study was to investigate the vitrification effect on Bax and Bcl-xl expression level in mouse metaphase II (MII) oocytes.

Methods: The survival and fertilization rate of MII mouse oocytes was assessed following vitrification by cryotop. Oocytes were randomly selected and distributed amongst the five experimental groups (control, docetaxel, docetaxel + VS (Vitrification solution), docetaxel + Vitrification, and vitrification). Then, vitrification method effect on the expression of Bax and Bcl-xl genes were determined in vitrified-warmed oocytes by real-time RT-PCR. Each group was compared with the control. Data were analyzed with ANOVA using GraphPad Prism.

Results: There were significant differences between Bax gene expression level in the control group with the docetaxel and

vitrification groups ($P < 0.05$). Bcl-x1 gene expression level was significantly high in the vitrification group (vitrification + Docetaxel) and non-vitrified group (Docetaxel + VS) ($p < 0.01$) figure 1 & 2).

Conclusions: This study indicated that the vitrification of mouse MII oocytes can increase the expression of apoptotic gene (Bax and Bcl-x1 genes). This result indicate a defensive mechanism of oocyte against vitrification.

Key Words: Vitrification, oocyte, Docetaxel, Bax, Bcl-x1

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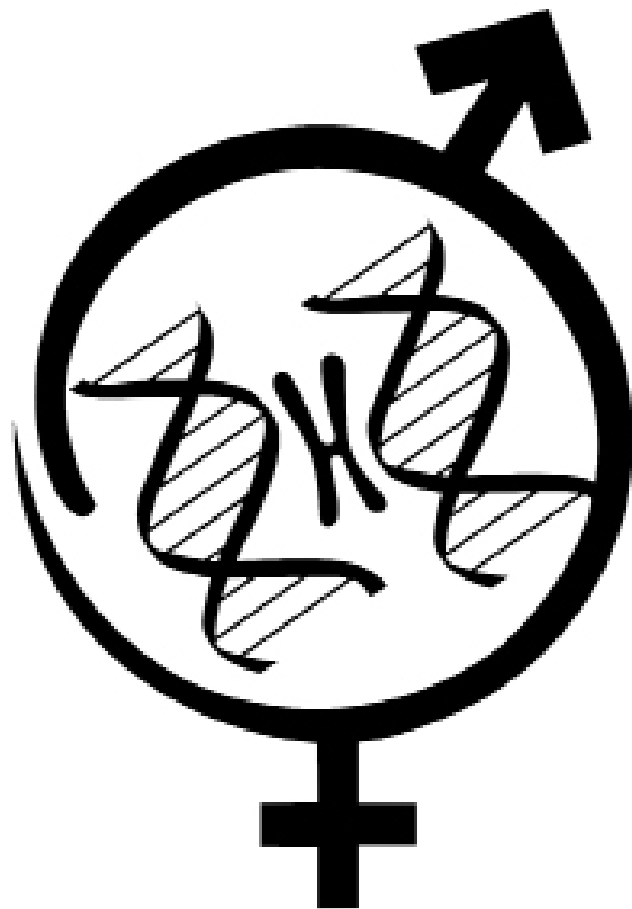
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Abstracts of
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Reproductive Biomedicine Research Center
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Invited Speakers

I_{nm}-1: Dilemmas in Providing Infertility Cre for High Risk Patients

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Fertility in patients with systemic disorder is commonly not violated. Women seeking infertility treatments if they are obese or have a strong family history of type 2 diabetes or are over the age of 40 should be offered a glucose tolerance test. Patients with systemic disorders, who are suffering infertility management, could be at risk of flare or thrombosis. SLE may alter fertility via an ovulation during episodes of active disease or chronic renal failure and using NSAIDs, high dose of corticosteroids and cyclophosphamide. Despite the increase in cardiovascular risk factors, morbidity and mortality from coronary heart disease among women with infertility has not been shown to be as high as predicted. The rate of live birth is decreased and maternal complications are increased in patients undergoing assisted reproductive techniques who suffer from systemic diseases. Clinicians should continue to identify systemic condition (including blood pressure, cholesterol, triglycerides and high density lipoprotein cholesterol) in women with infertility and treat these accordingly.

I_{nm}-2: Ovarian Somatic Stem Cells

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Reproductive gonads and tracts are expected to vastly remodel during mammalian life span, which lends credence to the essential role of somatic stem cell in regenerative cycle. Although there is a continuous debate about the existence of oocyte derived stem cell in adult ovary, the presence of somatic stem cells to produce different parts of ovary have long been neglected. The ovarian surface epithelium (OSE) and cortex mainly originate from somatic ovarian stem cells. OSE are composed of small number of cells that capable of self-renewing and differentiating into immature oocytes *in vitro* or *in vivo*. This quality, the capability of differentiation into two cell types and ovarian repair during ovulation, would apparently make OSE conducive to somatic stem/progenitor cell mediated processes investigation. Multipotent granulosa cell progenitors, as a source of somatic stem cells of ovarian cortex, can be differentiated into three distinct lineages and have a prolonged life span *in vitro*. The theca layer is another source of somatic stem cells in the ovarian cortex. Theca stem cells were isolated and purified from neonatal mouse ovary *in vitro*. In granulosa cell-conditioned media, these cells show signs of differentiation, lipid droplet accumulation, smooth endoplasmic reticulum formation and later, production of androstenedione, Luteinizing hormone (LH), Insulin-like growth factor 1 (IGF1), and stem cell factors (SCF). Similar to *in vivo* conditions, the transplanted theca cells moved into the mouse ovaries and were surrounded by the growing follicles. The cellular properties and the *in vitro* differentiation ca-

capacity of porcine ovarian theca-derived multipotent stem cells have also been examined. There is no information in literature, regarding human theca stem cells (hTSCs) and their behavior during cell culture. We have isolated and characterized hTSCs and differentiated into human theca progenitor cells (hTPCs) and human oocyte like cells (hOLCs).

Keywords: Human Mesenchymal Stem Cells, Human Theca Stem Cells, Human Theca Progenitor Cells, Human Oocyte Like Cells

I_{nm}-3: What Stem Cells Can Do for Infertile Couples?

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Germ cells (GCs) are responsible for fertility in humans. Disruptions in GC development or function cause infertility which is a major medical problem that affects 10-15% of couples worldwide. Current therapies for infertility are limited to *in vitro* fertilization and intracytoplasmic sperm injection techniques which are not applicable to infertile cases with few or no gametes. Therefore, advanced therapies should be explored for infertility treatment, which necessitates an in-depth understanding of GC development and function. However, low number of germ cells especially at the early level of development has been a limitation for studying GCs and mechanisms underlying reproduction. In this regard, derivation of GCs from embryonic stem cells provides an unlimited source of material to support the research for exploring principles that underlie reproduction as well as generate functional gametes for infertile couples.

Moreover, spermatogonial stem cells (SSCs) and oogonial stem cells (OSCs) that reside in testis and ovary respectively, are considered as germline stem cells that are important source for developing advanced therapies for fertility preservation that occur following cancer treatments. Importantly, OSCs have potential to be used for infertile women who have low quality oocytes that are failed in IVF cycles.

In conclusion, different type of stem cells has potential to develop advanced therapies for infertile couples that are not cured by current methods. However, more research are needed to apply them in clinic.

Keywords: Embryonic Stem Cells, Spermatogonial Stem Cells, Oogonial Stem Cells, Infertility, Reproduction

I_{nm}-4: Supporting Need for Couples after ART Failure by Nurse/Midwife

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Background: The complex nature of infertility and Long-term process of recurrent treatment cycles is stressful and demanding for couples in different ways regardless of their ethnicity, soci-

ety, culture or economic status along with tendency toward depression, anxiety and feelings of stress. If the treatment is failed the couple faces new choices such as whether or not to carry out a second or third treatment considering financial and economic constraints, bio-psycho-social and cultural dimensions. Nurses/midwives play a critical role in improving health outcomes, actively enabling decision-making within a positive relationship to solve their doubts and problems.

Materials and Methods: A Systematic Review was conducted.

Results: Although promotion of family support could suggest some help, the infertile couples preferred to have the suffering to themselves for fear of adding to their parents' burden. Support received from friends and relatives are minimal, but could be stressful and plays a limited role in the couples' efforts to cope with the hardships of treatment. Given the diverse nature of the clinical practice, administration of supportive intervention from health care providers are often described as inadequate due to the short consultation time in the clinic and the lack of psychological support. Nurses/Midwives as the professionals in the field of health and with regard to their important role in support, education, and care of patients and their caregivers, usually deliver safe services that contribute to support, educate, providing crucial follow-up and guide patients on success rates for different treatment options including the effect of the couple's background such as diagnosis and age, time of treatment, and termination of treatment in order to take steps toward encounter social, economic and physical barriers in addition to satisfy the information and psychological needs of outpatients through all stages of the journey of living with infertility. For couples who are unsuccessful in becoming pregnant and having a baby, cause stress and strain on the couple's relationship, the demands on counselling and information might be regarded as more important than for couples who achieve a pregnancy and have a child.

Conclusion: Despite the lack of research specifically examining the role and impact of qualified nurses and midwives at the heart of ART, they can play an important role in improving the quality of life and tolerability to the assisted reproduction treatment of patients undergoing ART through education and designing appropriate care programs, administration, and application of these supportive educational programs as well as safe, evidence based, appropriate, timely, efficient, effective & equitable care.

Keywords: Nurses/Midwives, Support, Assisted Reproductive Technologies (ART)

I_{nm}-5: Genetic Screenings before Conception and during Pregnancy

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Development of medical sciences in the field of reproduction

and fertility rises a lot of hope for birth of healthy children and prevention of congenital malformations. Genetics plays an important role in this regard, but collaboration and interconnection between obstetrician/gynecologists, perinatologists, midwives/nurses, geneticists and consultants is very important.

Responsibility of these groups will start from decision of couples to conceive or even before marriage. The role of obstetrician/gynecologists as the first who visit couples and midwives/nurses or general physicians in an optimal patient referring system, are most important. Suitable referring protocols and guidelines can avoid from unnecessary tests, which lead to more costs and more anxiety for couples.

Genetic diseases in the couples' relatives, make carrier testing through regular methods necessary. Alternatively, expanded carrier screening like exome sequencing, even reveal those late onset diseases that offspring may encounter in elderly age, which is a challenging issue.

Genetic work up of infertile patients is very important. Treatment team and especially genetic consultants must check genetic causes of infertility. Cases such as male infertility due to sever decrease or absence of spermatogenesis, recurrent implantation failures and recurrent abortions in this group of patients, must be noticed intensely.

Perinatal tests in the late first trimester or early in second trimester are so much important. How to select the tests such as maternal serum markers, sonography, cell free DNA present in maternal blood and the more invasive tests like Chorionic villus sampling (CVS) or amniocentesis is important duty of perinatologists. Does any of these tests are obsoleted? On the other hand, doing old procedure with new facilities like microarray for amniocentesis, does it provide information that is more precise? Are cfDNA test results definite?

What is the role of genetic consultants, nursing/midwifery in pre- and post-test counseling to increase the couples' ability to make informed decisions and reduce their anxiety? When there is need for intervention of psychologists?

Whether preimplantation genetic screening (PGS) has any role in achievement of infertile patients to a healthy birth, is yet under discussion among specialists. In the recent years, great developments take place in the procedure of PGS. These changes were made in whole chromosomes study and in the biopsy method. Are these changes improve the diagnostic value of PGS? Or extra data such as RNA sequences must be added to them?

The role of preimplantation diagnosis (PGD) in cases who couple is carrier of genetic defects is obvious, but the implantation of healthy embryos, is very important. Do these procedures move toward more comprehensive and precise methods?

The above issues and challenges especially those in the mind of audiences will be discussed in the panel.

I_{nm}-6: Obesity and Infertility

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The prevalence of obesity is increasing worldwide. Obesity has many negative effects on system performance and organs, including the human reproductive system. Obese women undergo disturbances of the hypothalamic-pituitary- ovarian axis, and

frequently suffer of menstrual dysfunction leading to anovulation and infertility. Moreover, in obesity the adipocytes act as endocrine organ. The adipose tissue indeed, releases a number of bioactive molecules, namely adipokines that variably interact with multiple molecular pathways of insulin resistance, inflammation, hypertension, cardiovascular risk, coagulation, and oocyte differentiation and maturation. Moreover, endometrial implantation and other reproductive functions are affected in obese women with complications including delayed conceptions, increased miscarriage rate, reduced outcomes in assisted conception treatments. Weight loss programs through lifestyle modification in obese women, have been proven to restore menstrual cyclicality and ovulation and improve the likelihood of conception.

In men, obesity and metabolic syndrome are known as an infertility factor. Three main biological mechanisms linking obesity to impaired male reproductive function: hypogonadism, testicular heat stress/hypoxia-induced apoptosis and endocrine disruption by environmental toxins.

With increasing the prevalence of obesity and its impact on the fertility of women and men, infertility treatment team, needs to better understand the pathogenesis mechanisms for more awareness and develop effective ways of preventing and treating obesity.

I_{nm}-7: Managing Assisted Reproduction in Women of Advanced Age

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There are significant ethical considerations and medical (maternal and fetal) complications related to pregnancy in peri- and post-menopausal women.

Female infertility, pregnancy loss, fetal anomalies, stillbirth, and obstetric complications are more common in advanced reproductive age.

Oocyte donation reverses the age-related decline in implantation and birth rates of women in their 40s and 50s and restores pregnancy potential beyond menopause.

The risk of medical complications can be mitigated by careful medical screening of the mother and the use of ARTs in healthy women. In these instances, a woman of advanced maternal age who is otherwise healthy can carry a pregnancy with a similar risk profile to that of her younger counterparts when using donated oocytes. However, obstetrical complications in older patients remain high, particularly related to operative delivery and hypertensive and cardiovascular risks. A thorough medical evaluation designed to assess the physical fitness of a patient for pregnancy before deciding to attempt transfer of embryos to any woman of advanced reproductive age (>45 years). Embryo transfer should be strongly discouraged or denied to women of ARA with underlying conditions that increase or exacerbate obstetrical risks. Because of concerns related to the high-risk nature of pregnancy, as well as longevity, treatment of women over the age of 55 should generally be discouraged.

I_{nm}-8: Feasibility of Menstrual Blood Stem Cells in Cell Therapy of Reproductive Disorders

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Nowadays, the menstrual blood has been introduced as a non-invasive source of mesenchymal stem cells (MSCs) with several advantages such as easy accessibility without need for an aesthetic, renewability as they can be sourced on a monthly basis, high proliferative capacity in culture without inducing genetic abnormalities, and non-ethical concerns. We showed that isolated menstrual blood stem cells (MenSCs) have higher proliferative and self-renewal capacities than other MSCs like bone marrow stem cells (BMSCs) and can modulate the inflammatory reaction. Moreover, MenSCs could differentiate into multiple mesodermal and occasionally endodermal and ectodermal lineages. Besides efficiency of MenSCs in treatment of non-reproductive disease, there are some evidence implying restoration ability of MenSCs in female reproductive disorders like Asherman disease and premature ovarian failure. These data make MenSCs extremely attractive and useful for stem cells therapy even in allogeneic cellular therapies and open a wide perspective of potential clinical applications of reproductive disorders to other stem cell sources. At present, some clinical research groups and companies launched clinical trials using these cells. However, to translate the results of in vitro and animal studies into clinical phase, the safety of MenSCs administration should be assessed in long-term pre-clinical and large-scale clinical studies. Another interesting idea is MenSCs banking in order to treatment of probable disorders in especially post-menopause age. Since these cells are well tolerated, with no toxicity or any adverse side effects report, banking and holding this source of stem cells can be suitable for clinical use. Thus, it sounds that MenSCs banking has a vast scope in future and is the next big thing in the medical world. Beside clinical application, the possible role of MenSCs as a diagnostic tool especially in the pathogenesis of endometriosis, endometrial hyperplasia and endometrial cancer has been suggested. These finding can be achieved through the study of MenSCs obtained from women with pregnancy disease in comparison to MenSCs isolated from donors with uncomplicated pregnancy history. Future research and new evidence would greatly propose MenSCs as a novel and best diagnostic tool in complicated pregnancy. Therefore, MenSCs are extremely attractive and useful for diagnosis of reproductive disorders and also stem cells therapy even in allogeneic cellular therapies and open a wide perspective of potential clinical applications to other stem cell sources.

Keywords: Menstrual Blood, Stem Cell, Differentiation, Cell Therapy, Regenerative Medicine, Clinical Application, Reproductive Disorders

I_{nm}-9: Three Neglected Concept in Empathy

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Psychic distance is one important topic in aesthetics and artistic criticism that is also related to empathy. In aesthetics psychic distance's meaning is that art have distance with us to create aesthetics entente, and do not be confused with reality. Omitting

psychic distance and deep sympathy and psychological with art, prevents artistic judgments and aesthetics approach. In medical ethics it is important while encountering patients. Reduction of psychic distance and excessive sympathy with patient prevents ordering of physician-patient relationship as a important element of treatment.

One of the basic concepts of phenomenology is attainment of phenomenon intentionality. So that recognizer parentheses all his identified assumptions (Epoche) and attended the current visibility status that obtained. Some thinkers like Franz Brentano, know first sight rich of intentionality and phenomenology approach. For instance the first sight at a pleasing landscape raises us sort of significant that may nothappen at next encounters. Because next encounters are full of different defaults of that matter and also falling into the habit may happen to intentionality. It seems that doctor-patient relationship have a significant correspondence with phenomenology approach. Doctors must correct and expurgation defaults toward patients and also must defend the intentionality and not to fall into the habit.

are two ways moral sensitivity. First, strengthening phenomenological approach by renewing the first sight, each time subject re-identification (patient for instance) should priority attended and increasing moral sensitivity. Second, assuming that each situation could be fork or ethical conflicts think of adverse impact of matter and as result look at every matter as an ethical perspective. However at commencement this important matter has overlap with maximal ethics but especially in heterogeneous communication such as doctor-patient relationship have an especial situation. It seems upgrading moral sensitivity is the target of phenomenology of ethics in doctor-patient relationship matter

I_{nm}-10: Support Group for Infertile Couples

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Support group is a gathering of people with same problem or disease where they can talk and share their experiences. In these meetings, coping strategies are discussed and people feel more empowered by joining support groups. Normally, experts do not interfere in these sessions and only involved people share information. Being in same position helps people not feeling any judgment or stigma, meanwhile have a good feeling of being understood by other people with the same problem. These groups are mostly experienced in people with chronic or life threatening diseases like cancer, but less experienced in short term treatable diseases like infertility. Unfortunately, infertility is a big stigma in our society and also in other middle Eastern countries and includes some difficult treatments which are not well accepted like donation programs and surrogacy. Dealing with such conditions are not easy and experiences presented by people with same disease helps specially those who were successful. It can reduce stress and anxiety, brings hope and self-esteem and help the patients making their decision about difficult situation. The best activity of these support groups are in cases of treatment failure who are prone to lose their hopes and think of not continuing their treatment. Infertility clinics should provide space and equipment for support groups without interference in their programs or discussions and leave them to

talk freely together but an expert must be ready to answer the questions or correct some incorrect attitude mostly by request. Management of the session should be on the audience but invitation of successful patients especially hard cases and also mellifluous people are highly recommended. In some sessions law experts, social workers or religious scholars can be invited by demand. It is concluded that support group is very useful for infertile couples which can reduce psychological and social pressure and also can help patients make their decision and continue their treatment.

Keywords: Support Group, Infertility, Failure, Psychological, Stigma

I_{nm}-11: Break Bad News

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Talk about secret information is one of the most serious responsibility for medical staffs, In spite of our choosing, we should transfer many bad news to our patients.

Understanding the knowledge and skill for break bad news is obligation for staff, so these messages would have negative effect to person's mind.

Definition: Any information which adversely and seriously affects an individual's view of his or her future.

Why is it important: It can affect the patient's understanding of information, and future decision.

Six step strategy for Breaking Bad news:

1. Setting up the interview
2. Assessing the patient's perception
3. Obtaining the patient's invitation
4. Giving knowledge and information to the patient
5. Addressing the patient's Emotions with empathic responses.
6. Strategy and summary

I_{nm}-12: Oncofertility: Preserving Fertility in Patients with Cancer

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Background: Cancer can be a distressing diagnosis. Particularly, malignancy and its indicated chemo- and radiation therapies have extremely adverse impacts on the fertility of young cancer patients.

Materials and Methods: A comprehensive literature search was performed with suitable studies identified through the searching of electronic databases, alongside the screening of relevant reference lists on oncofertility support needs for cancer patients of reproductive age.

Results: Fertility is often a major issue for cancer survivors, With recent approaches to cancer care, survival rates have enhanced; therefore, the health care team has a responsibility to provide education for these patients and discuss their options and concerns regarding the effects of the cancer treatment and

the treatments available for the preservation of fertility so that an informed decision can be made as promptly as possible, and have a team ready to preserve fertility once a decision has been made as well as to reduce significant later life impacts and meet the support needs of cancer patients and survivors to achieve better health outcomes and improve their wellbeing. Cancer patients hold concerns regarding their fertility during the entire cancer journey, from diagnosis, throughout treatment and into survivorship and at each of these time points, their concerns and consequent care needs may change depending on patient age, gender, or child-bearing status. The oncologist responsible for the treatment of malignancy should consider the awareness of cancer patients of reproductive age (14-45 years of age), as part of the management and inform them of the possibility of complications induced by chemotherapy, radiation therapy and surgery for future fertility and techniques to conserve fertility. Cryopreservation of a woman's gametes and gonadal tissue may involve oocyte vitrification or ovarian tissue banking/transfer embryo, with or without ovarian stimulation, and ovarian transposition (OT). Currently oocyte and embryo banking are the standard procedures used for young patients with cancer whose future fertility is under risk.

Conclusion: As usual human emotions are consistent, universal and unchanging; it is clear to everyone that the emotional support of patients prioritize dealing with them for their recovery. Equilibrium in the role of disease and its acceptance for the patients makes it possible for them to overcome the disease and consider it as part of their life experience.

Keywords: Fertility Preservation, Cancer, Oncofertility, Oncology, Patient Needs, Emotional Support

I_{nm}-13: The Role of Cord Blood Preservation in The Hematopoietic Stem Cell Transplantation

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It has been for more than half a century that hematopoietic stem cells have been applied as a treatment method for the patients suffering from blood disorders. The source of this treatment method is originated in bone marrow in which bone marrow hematopoietic stem cells is derived from a healthy person and transferred to the patient, providing both sides are found matched genetically. Since this is an intervention method, the operation will need to be done under anaesthetic and hence there may be some negative side effects and risks. There is also found another source for hematopoietic stem cells which is called peripheral blood which can be obtained through motivating the stem cells to be removed from bone marrow and enter in peripheral blood. The ultimate accessible source to hematopoietic stem cells comes from cord and embryo which used to be considered as biological waste and were thrown away after the baby birth. This blood is rich in stem cells and through cryopreserving it the transplantation centers will have a permanent access to a source of stem cells which can be exploited at the time of need for ordinary or unremitting blood diseases.

Following the first transplantation taking advantage of cord blood in 1988 by professor Gluckman which was performed on a patient suffering from Falconi Anemia, we have witnessed more than 35000 more transplantations performed all around

the world applying cord blood units. All such transplantations were performed on the patients who had no appropriate or match donor and cord blood was found to be their only treatment choice to save their lives.

Cord blood cryopreserving happens in the form of either public or private banks and so far there have been about 700,000 units cryopreserved in public banks and some 4,000,000 units in private ones all over the globe. It should also be stated that cord blood stem cells can be used to treat some other diseases which are not concerned with blood such as CP, Autism, Diabetes Type One, Stroke, Dystrophy and so forth; though these methods are still under clinical trial phase.

Oral Presentation

O_{nm}-1: Comparison of Marital Satisfaction in Infertile Couples and its Relationship with Infertility Related Stress

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Background: Infertility is a personal and social issue that affects couples with multiple psychiatric disorders and impact on marital life and family functioning. Marital satisfaction is a true sense of satisfaction with marital relationship that can affect the physical and psychological aspects of couple life. This study aimed to investigate marital satisfaction in infertile couples and its relationship with infertility related stress.

Materials and Methods: In this cross-sectional study, 150 infertile couples visiting infertility centers in Tehran, Iran, were selected using the convenience sampling method. To collect data, a demographic form, ENRICH Marital Satisfaction Scale, and Fertility Problem Inventory were employed. To analyze the data, we used Paired T test, Pearson correlation coefficient, and stepwise regression in SPSS, version 18.

Results: Paired t-test showed a significant difference between the mean marital satisfaction scores in couples. The average marital satisfaction score was higher in men (P=0.003). The mean scores of couples in the sub-scales of "marital satisfaction" and "idealized distortion" had a significant difference (P=0.003, P=0.006). The result of linear regression test showed a significant relationship between marital satisfaction and infertility related stress (P<0.001).

Conclusion: Infertility stress in both men and women can affect the satisfaction of their marital relationship. Therefore, special attention to stress management counseling and control in this group of couples can be very effective.

Keywords: Infertility, Stress, Marital Relationship, Marital Satisfaction

O_{nm}-2: Development and Psychometric Properties of The Decision-Making for Reproductive Donation Questionnaire in Iranian Infertile Couples

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Background: Although many infertile couples have to decide about choosing reproductive donation or not, no predictive scales exist for evaluating the process of decision-making about whether or not to choose reproductive donation and the

determinants of this decision in this group. The present study was conducted to develop a decision-making questionnaire for selecting reproductive donation (DMRDQ) and assesses its psychometric properties in Iranian infertile couples.

Materials and Methods: This scale development study was conducted based on De Vellis' method (2012) in four steps at Milad Fertility Clinic in Mashhad, Iran. The dimensions of the concept of decision-making were determined in the first step based on the qualitative results obtained from 38 semi-structured in-depth interviews with nine couples and 14 infertile women applying for reproductive donation and a number of key people, including two gynecologists, two midwives and two clergymen. Items appropriate for the questionnaire were developed in the second step using the qualitative data and a review of literature. In the third step, the research team reviewed and reduced the items. The fourth step was dedicated to evaluating the face, content and constructs validity (through exploratory factor analysis) and the initial and final reliability of the questionnaire on a sample of 220 infertile women.

Results: Based on the results of the qualitative study, a pool of 170 items was developed, 101 of which were eliminated after revision due to being ambiguous or repeated or due to their poor face and content validity and initial reliability. The questionnaire was evaluated for its construct validity with 69 items. After the exploratory factor analysis, the DMRDQ was finalized with 51 items and seven factors, including the role of social networks, coping strategies, the decision to disclose or conceal, interpersonal relationships, religious quests, donor's characteristics and challenges in the process of treatment. All the factors had Cronbach's alpha values of 0.75-0.87 and intra-class correlation coefficients (ICC) greater than 0.7.

Conclusion: This study led to the development of a valid and reliable scale for examining infertile couples' decision-making about whether or not to use reproductive donation. The DMRDQ can help infertile couples with this decision by assessing the factors affecting the process of decision-making.

Keywords: Decision-Making, Reproductive Donation, Scale Development, Psychometric Property

O_{nm}-3: Aloe Vera Decreases Male Rat Fertility *In Vivo*

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Background: The pharmacological actions of aloe vera, as studied in vitro or in animals, include anti-inflammatory and anti-arthritis activity, and antibacterial and hypoglycaemic effects. Aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. The current work was undertaken to investigate the validity and/or invalidity of the aloe vera on enhancing the reproductive activity in male rat.

Materials and Methods: Thirty three adult male rats were divided into three groups. Experimental groups received aloe vera orally for 60 days in two different sublethal doses; 100 mg/kg as high dose and 50 mg/kg as low dose, whereas the control

group received distilled water.

Results: The administration of the aloe vera result did not show any significant difference in the weight of the seminal vesicle, liver and kidney of the treated groups relative to the control ($P \geq 0.05$). On the other hand, the results shown a significant decrease in the body weight of both the low and high dose-receiving groups in comparison to the control group. The extract of this plant caused a decrease of the following in the two experimental groups, compared to the control group: sperm count, motility and normal morphology, pregnancy rate and diameter of seminiferous tubules. Also, distortion of morphology of the seminiferous tubules and arrest in spermatogenesis was observed in the experimental groups.

Conclusions: From the present study, we can conclude that aloe vera acts as an anti-fertility agent.

Keywords: Aloe Vera, Seminiferous Tubule, Sertoli Cells, Testosterone

O_{nm}-4: Comparing The Effects of Palm Pollen with Letrozole+ Tamoxifen in Infertile Woman with Polycystic Ovary Syndrome on Pregnancy Rate

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Background: polycystic ovary syndrome (PCOS), one of the most common causes of infertility due to anovulation, affects 4-7% of reproductive women. The aim of this research is to compare the effect of palm pollen with letrozole + tamoxifen on pregnancy rate as treatment of infertile polycystic ovary syndrome women in Jahrom city.

Materials and Methods: This comparative clinical trial was done on 30 infertile PCOS women referred to Dr.rasekh clinic with aged 18-42 years. patients were randomly allocated to either case or control group. The control group prescribed letrozole + tamoxifen and Case group palm pollen from third to eighth day of menstrual cycle. Transvaginal ultrasound parameters including Ovarian follicular size, numbers and endometrial thickness were measured during treatment and based of this parameters continue these regimens and prescribed trigger drug.

Results: Endometrial thickness in palm pollen groups and tamoxifen+letrozole groups had significant difference in first visit ($P < 0.019$) respectively but no significant difference in the second and third visit in two groups. In the first visit, there was a significant difference in the size of left ovarian follicles in case and control group ($P < 0.004$), but in the second and third visit, the left ovarian follicle size in case group was larger than control group, without significant difference statistically. In the first visit, there was a significant difference in the size of right ovarian follicles in case and control group ($P < 0.001$) but, no significant difference in second and third visit. Pregnancy rate was higher in the group using palm pollen but there was no significant relationship between two groups ($P < 0.624$).

Conclusion: Because of significant effects of palm pollen on increase of endometrial thickness and size of dominant follicle, and eventually increase of pregnancy rates. Therefore, we rec-

ommend, this low costs, low side effect regimen in treatment of PCOS patients.

Keywords: PCOS, Palm Pollen, Letrozole, Tamoxifen, Pregnancy Rate

Poster Presentation

P_{nm}-1: A Review of Gamete Donation from a Legal Perspective

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Background: Approximately 10-15% of couples in Iran suffer from infertility. In a number of cases, they have no choice but to use a Donate gamete for treatment. In many societies, religious values are considered as influential factors in people's decision making. In Iran, according to the law passed by the Islamic Consultative Assembly in 1382, the donation of gametes is permitted under certain conditions for some great authorities therefore, knowing the issues of the Shari'a must be prioritized. The legal process in the donation of the titles does not have a lot to do with this article, which deals with the topic of the donation of the gamete.

Materials and Methods: A review and method of collecting data from Islamic texts and Fatwa of the jurists.

Results: In case of a couple's gametes be used provided that the unlawful act such as touching and masturbation do not consider it practical jurists permissible. If there is a different use of the foreign gamete, which is often forbidden by it. The adherents have different opinions in the assignment of a foreign gamete, but given the fact that the Supreme Leader permits permission to do so in their own right, and given this the important issue is social and governance issues in such a task is referred to the supreme Leader's fatwa.

Conclusion: Therefore, the chosen point is the same fatwa of some jurisprudents who have said: the artificial insemination of a woman through the spit of a barbarian man in itself is not a problem, but it should be avoided from the forbidden preambles, such as the look and the touch of haram and others. However, if it is born with this method of childhood, it does not belong to the woman's husband, but to join the owner of the spleen and to the woman who owns the womb and the egg.
Keywords: Donate The Gamete, Assertive Sentence, Legal Issues

P_{nm}-2: Conflict Resolution Strategies and its Relationship with Marital Satisfaction: Iranian Infertile Couples

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Background: Marital satisfaction implies the extent to which couples' expectations are met, and its reduction can have adverse effects on couples' physical and mental health. Infertility affects marital satisfaction by creating conflict in marital relationship. In this study, we sought to investigate conflict resolution strategies in infertile couples and their relationship with

marital satisfaction.

Materials and Methods: In this cross-sectional study, 150 infertile couples attending infertility centers in Tehran, Iran, were selected using the convenience sampling method. To collect data, a demographic form, ENRICH Marital Satisfaction Scale, and Rahim Organizational Conflict Inventory-II were employed. To analyze the data, we used measures of central tendency and dispersion, Pearson correlation coefficient, and stepwise regression in SPSS, version 18.

Results: The most and least frequently adopted strategies were the collaboration and compromise strategies. In men and women, the collaboration strategy predict marital satisfaction (P<0.001).

Conclusion: In infertile men and women of this study, the collaboration strategy positively predicts marital satisfaction. Therefore, couples' strategies should be identified in counseling sessions using standard tools to modify inappropriate strategies. Further qualitative studies are recommended to identify the factors influencing the use of appropriate and inappropriate strategies.

Keywords: Marital Conflict, Conflict Resolution Strategies, Infertility

P_{nm}-3: The Role of Lifestyle Factors on The Reproductive Health

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Background: The lifestyle factors can greatly influence overall health and well-being, including fertility. Fertility is affected by factors such as age, nutrition, weight, exercise, and psychological stress. Some factors have a negative impact on fertility such as alcohol and caffeine consumption, smoking and illicit drug use. The aim of this study is to review the role of lifestyle factors in infertility men and women.

Materials and Methods: This review was carried out by using of different sites of internet and considering the studies which were published in 2011-2017 and included if they contained data on lifestyle factors on the reproductive health.

Results: Age of a man or woman is a factor among others that can affect fertility. With the increasing of age in men, testosterone levels begin to decrease, Semen parameters also begin a steady decline, semen volume and motility both decrease, and morphology may become increasingly abnormal. With the increasing of age in women, the number of oocytes is declined, menstrual cycle is shortened, chromosomal abnormalities and aneuploidy are increased. The diet rich in carbohydrates, fiber, and folate as well as fruit and vegetables correlates with improved semen quality. Women who take multivitamins may be less likely to experience ovulatory infertility. Other findings will be presented in full text of paper.

Conclusion: Age, nutrition, weight management, exercise, and psychological stress, may affect fertility. The evidence suggests that age may play a large role in determining fertility. The greatest chance of success for fertility is before the age of 30 for women and before 35 for men. Recent studies suggest that weight plays an important role in fertility and maintaining an

ideal weight may provide a way for couples to increase their fertility.

Keywords: Lifestyle Factors, Reproductive Health, Infertility

P_{nm}-4: Failed Assisted Reproductive Technologies: Psychological Outcomes

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Background: The methods of assisted reproduction are an important option for those looking for help to conceive and are well-established. Despite increases in treatment application, its success rate is not high. The factors which associated with the lower success of assisted reproductive technologies (ART) are: duration of infertility, increasing number of ART cycles and increasing maternal age. The aim of this study was to review the psychological outcomes associated with failed ART treatment in men and women.

Materials and Methods: This review was carried out by using of different sites of internet and considering the studies which were published in 2010-2017 and included if they contained data on psychosocial outcomes before and after ART treatment.

Results: Depression and anxiety increase after the treatment failure of assisted reproductive technologies and a failed ART treatment was positively associated with depression and anxiety in both men and women, but depression and anxiety decrease as time passed from ART procedure.

Conclusion: Psychological adverse outcomes increase after the treatment failure of assisted reproductive technologies. A more comprehensive decision-making framework for health policy around ART practice is needed.

Keywords: Assisted Reproductive Technologies, Psychological States, Failed Treatment

P_{nm}-5: Reasons of Becoming A Surrogate Mother, Psychological Issues and Social Attitudes in Iran: A Review Study

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Background: Surrogacy is one of the methods of infertility treatment. This method is applied in the cases where there is no possibility of pregnancy for women due to medical reasons, including congenital malformation, hysterectomy, postpartum hemorrhage or menorrhagia, repeated failure after IVF, recurrent abortion and contraindicated in pregnancy. Although surrogacy has been applied in Iran, it has been caused the psychological and social problems in surrogate mothers. The aim of this study was to investigate the reasons of becoming a surrogate mother, psychological issues and social attitudes toward them in Iran.

Materials and Methods: A thorough research was carried out using keywords such as "surrogacy", "Reasons of surrogacy", "social stigma", "social attitude", "surrogate mother", "and host mother" and "in Iran". The data were collected from Google Scholar and Google English and Persian language articles.

Results: According to literature, the reasons of being a surrogate mother in Iran were respectively included financial demands (receiving money), to help relatives and friends to become mothers, humanitarian motivation, and spiritual motivation. Totally, the general population did not have a positive attitude toward the surrogate mother; however, in some studies, most fertile women had a positive attitude toward the surrogacy. Surrogacy has caused complicated social and psychological problems for the surrogate mother in Iran. The emotional bond to the fetus, increased risk of anxiety, postpartum depression, to feel angry and sinful, adverse psychological effects on the wife and children of the surrogate mother, negative attitudes of friends and relatives, and their humiliation and misconduct caused psychological problems on the surrogate mothers. Although in one study, most surrogate and intended mothers did not consider surrogacy a problematic issue. The most important concerns of the surrogate mother were included: awareness of relatives, how to conceal the pregnancy (especially in single women), and how to explain the absence of baby after delivery which makes them in the psychological pressure.

Conclusion: The lack of social support of surrogate mothers has provided the conditions for future vulnerabilities. Surrogacy should be considered as the high-risk psychological experience in Iran. This review reveals the need to inform the surrogate mothers and her families about the details and problems of the surrogacy method, a careful and continuous counseling along with the support of the expert psychologist team during the process of surrogacy. Also, the media especially the radio and television programs, and social networks can play the positive role in the awareness and changing the attitude of the community toward the surrogacy.

Keywords: Surrogacy, Reasons, Psychological Factors, Social Attitude, Surrogate Mother

P_{nm}-6: Review of Knowledge, Infertility Knowledge and Educational Needs from Infertile Viewpoints

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Background: Infertility is defined as the inability of a couple to become pregnant, after a year of sexual intercourse without contraception. Awareness of risk factors for infertility is the first step in maintaining fertility in the future. For empowerment, reduce health care costs and provide high-quality infertility care based on patient needs, it is necessary to identify and meet the educational needs of infertile people. So, this study aimed to review the knowledge and knowledge of infertility and educational needs of infertile people. Articles published in English and Persian with the same population as the study population were included.

Materials and Methods: Academic and step-by-step searches for the purpose of studying the PubMed/Medline, Google Scholar, Web of Science and SID Magiran databases were reviewed during 2013-2017. In first 147 articles were found and finally 14 papers were selected based on inclusion criteria. English and Persian articles with the same population and aim were included. Articles that focused exclusively on theories, case-report, were excluded.

Results: There is a poor knowledge of hygiene in two genders (3.08 ± 0.99) this knowledge gap correlates with the delay in birth and infertility treatment. Most women believe that when they first understand their problem, they must receive fertility education (92.2%). The greatest barriers to educating fertility awareness, short-term counseling and time limitation of general practitioners and the lack of educational materials needed for infertile persons. Most patients (92.1%) and men (83.3%) believed that infertility education programs should include the causes of infertility and different types of diagnostic and laboratory treatment methods, because, knowledge of the causes and methods of treatment of infertility was very weak. Post-treatment education was also inadequate for patients (70%). In order to reduce the risk of burnout for infertility and increase the success rate of infertility treatments by correcting these risk factors, lifestyle health education was also effective (46.1%).

Conclusion: Considering the health evolution map in Iran and adopting a new population growth approach, and given the increasing incidence of infertility, it is necessary to seriously investigate the situation and determine the priorities of infertile society based on their needs. Adult education in reproductive age and the population of infertile patients can empower them. Understanding the needs of infertile couples can be an effective step in solving problems and reducing their psychological stress, and also by organizing infertility centers to increase their productivity. It seems that developing a standard curriculum for clinical infertility patients that includes patient priorities and knowledge gaps to be necessary. Therefore, create of appropriate, efficient and targeted policies based on need approach on the target group in reproductive health need for mental health, increased well-being and promotion of health.

Keywords: Infertility, Education, Training, Knowledge

P_{nm}-7: Psychological Effects of Fertility Problems

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Background: Infertility can be very stressful. The psychological and physical trauma associated with investigation and treatment can often be exacerbated by the length of treatment and the multi-disciplinary approach that is involved.

Materials and Methods: This is a review article. Literature for the period 2005-2018 was searched in the electronic databases of Google scholars, Cochrane, science Direct and Pub Med using the following key words.

Results: The relationship between psychological stress and fertility problems is complex. The psychological state of couples undergoing IVF may vary at different stages of treatment, the most stressful stages being waiting for the outcome of treatment and finding out that IVF has been unsuccessful. Four surveys have reported that most patients feel that access to a support group and counseling would be beneficial. Some felt that psychological support should be available at all stages of infertility treatment and investigation. In another study, 70% of patients said they would request counseling if it were available free of charge. Despite this, overall uptake of counseling is low at between 18% and 25%. It has been suggested that less distressed patients may not wish to receive counseling, and some may cope

well with support from their spouses and family. Two-thirds of patients undergoing IVF treatment reported reading newspaper or magazine articles and watching television programs about the psychological aspects of infertility, even though few participated in a support group or sought counseling before treatment. This suggests that, for some patients, information about local and national support groups and booklets on the psychological aspects of treatment, in addition to medical information, may be beneficial.

Conclusion: The emotional consequences of anxiety and stress can be reduced by adequate provision of clear information about all aspects of investigations and treatment, involving both partners as an integral part of the management plan. Counseling involves a professional relationship between a qualified counselor and a patient, who may be an individual, a couple or a group of people. This relationship is contained within a formal counseling contract agreed and understood by both parties. The counselor has no other relationship with the client. Nurses, doctors and scientists in fertility clinics offer support and emotional help to couples as part of their professional role, but it is necessary to recognize this as using counseling skills within an existing role.

Keywords: Infertility, Psychological Treatment, Counseling

P_{nm}-8: Association between Serum Folate and Vitamin B-12 Outcomes of Assisted Reproductive Technologies

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Background: Assisted reproductive technologies (ARTs) which include in vitro fertilization and intracytoplasmic sperm injection, have become the main treatment modalities for couples facing infertility. Preconceptional folate and vitamin B-12 have been linked to beneficial reproductive outcomes in both natural pregnancies and those after ART treatment.

Materials and Methods: This is a review article. Literature for the period 2005-2018 was searched in the electronic databases of Google scholars, Cochrane, science Direct and Pub Med using the following key words.

Results: The suggestion of an interaction between folate and vitamin B-12 on outcomes of ART has not been previous evaluated; however, biological rationale supports this finding. Vitamin B-12 is a cofactor for folate-dependent methionine synthase, which is involved in homocysteine remethylation. The methionine derivative S-adenosylmethionine is the most important methyl donor in the body for the methylation of lipids, proteins, and DNA. A deficiency in S-adenosylmethionine reduces DNA methylation and consequently leads to hypomethylation of DNA, which may lead to aberrant patterns of gene expression. Synthesis, repair, and methylation of DNA are crucial in gametogenesis, fertilization, and pregnancy. Another consequence of impaired methionine synthase is the accumulation of homocysteine, which may induce cytotoxic and oxidative stress and lead to impaired oocyte maturation, embryo development, and endothelial cells. Exposure of trophoblast cells to elevated homocysteine may also increase cellular apoptosis and lead to inhibition of trophoblastic function, which is essential for successful placentation. Nevertheless, whereas live birth rates were

highest among women with high serum folate and vitamin B-12 concentrations, the interaction was not statistically significant and the study was underpowered to identify interactions.

Conclusion: Higher serum concentrations of folate and vitamin B-12 before ART treatment were associated with higher live birth rates among a population exposed to folic acid fortification. In conclusion, researchers found that high concentrations of folate and vitamin B-12 in serum are associated with an increased chance of live birth after ART. These findings support the importance of preconception folic acid supplementation and suggest the additional intake of vitamin B-12. Given that live birth rates per initiated ART cycle have plateaued for approximately a decade in the United States, a randomized trial of high-dose supplementation with folic acid and vitamin B-12 before planned ART warrants serious consideration.

Keywords: Vit. B12, Folate, ART Treatment

P_{nm}-9: ART and Ultrasound

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Background: The aim of this study is to investigate the role of ultrasonography in female infertile patients. Every patient in the process of ART needs to be followed-up and based on the gathered data the best treatment should be chosen.

Materials and Methods: A narrative review was performed within articles published in "PubMed", "Elsevier", and "SID" as well as some original text books to reach the objective.

Results: When a patient wants to start the treatment must roll out disorders in order to opt for the best treatment method and to manage the correct scan for the right patient. As Uterus: anomaly, adhesions, polyps, myomas, adenomyosis, Cervix: anomaly, function. Tubes: Occlusion, hydrosalpins, pyosalpinx. Ovaries to check up antral follicles, ovulatory cycles, LUF, PCO. After all, when the method of treatment cleared, the most important effect of sonography is in treatment cycles such as: stimulation of ovaries, checking the endometrial thickness, follicle aspiration, embryo transfer and checking the improvement of pregnancy. As we know pregnancies following IVF are associated with higher risk of obstetric morbidities and perinatal mortality. Several studies have demonstrated that the rate of prenatal complications is significantly more frequent in IVF-conceived pregnancies compared to spontaneous pregnancies.

Conclusion: Thus, a proper sonographic evaluation is required in these patients. Sonography is known as the first imaging modality in the investigation of the female pelvis. Ultrasound has a pivotal role in imaging modality in the study of the female pelvis, and provides fundamental information in detecting uterine, ovarian, or adnexal origin, but to reach to the best results we need to use suitable procedure.

Keywords: Ultrasound, ART, Treatment

P_{nm}-10: Prenatal Care after Assisted Reproductive Technologies

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Background: With advances in assisted reproductive technologies (ART), the numbers of women looking for infertility services are increasing. Of all pregnancies conceived after ART treatment, 63% is conducted by Intracytoplasmic sperm injection (ICSI) and the use of this procedure is continuing to increase. Results of follow-up studies on children born after ICSI shows an increased risk of congenital malformations, preterm birth and obstetric complications in ICSI and IVF singletons compared with naturally conceived children. However, it is suggested that these risks may be associated with pre-existent female medical conditions, age-related factors or diagnostic and operative procedures of infertility management. In line with these results, it is expected to consider more carefully antenatal care in these mothers. This article provides an overview of prenatal care after ART.

Materials and Methods: A literature search was conducted through PubMed

Results: Many ART patients are worried about the possible loss of their pregnancy and this might result in a very serious prenatal care of these patients. The high number of ultrasound examinations and health care service visits are indicative of the doctors' efforts to evaluate these pregnancies carefully. The data of a prospective controlled multicenter study report that longer mean duration of antenatal hospitalization and higher costs of health care in the IVF mothers compared to the spontaneously conceived singleton pregnancies may be due to the higher risk of preterm labour and bleeding during pregnancy. According to studies, consumption of supplementary iodine and periconceptional folic acid is found to be inferior to standard in patients under ART. Data on the use of vitamins and medications in pregnancies after ART is rare. Smoking and drinking alcohol in pregnancy leads to harmful effects on the fetus which are irreversible with no therapeutic options and the only therapy is prevention. Regular exercise during pregnancy can improve physical fitness and can have multiple health benefits for mother and fetus.

Conclusion: More ICSI mothers use the facilities of antenatal care with regular prenatal visits and with systematic ultrasound examinations as compared to those who conceive spontaneously. The attention ought to be paid to counselling, and ICSI mothers should be well informed about the necessity of: prophylactic use of folic acid, use of iodide supplementation during pregnancy, quit smoking and alcohol consumption before pregnancy and avoiding illegal drug abuse during pregnancy.

Keywords: Prenatal Care, Assisted Reproductive Technologies, Intracytoplasmic Sperm Injection

P_{nm}-11: The Benefits of Using Complementary Medicine on Infertility: A Review Article

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Background: Infertility affects roughly 15% of couples. Various therapeutic factors are used to help these patients, none of which are definitive. Research has shown that lifestyle changes, Complementary and Alternative Medicine (CAM) are effective in the fertility rate. Reasons for using CAM vary and may include faith, beliefs, economic factors or simply fear of a surgical procedure and apprehension about using hormone-modifying medications. This study reviews the role of complementary medicine in improving infertility.

Materials and Methods: In order to investigate some of the nutrient superstars on infertility which have been studied to date, keywords including Infertility and complementary medicine was searched in the following databases: ISI, Pub Med, Science direct, Google Scholar, Scopus, Science direct, Iran medex and Magiran from 2000 to 2017 in which 25 papers were evaluated.

Results: Novel studies give a different view of infertility proving that nutrient levels, diet and lifestyle choices can be huge factors in infertility. Follicular nutrients may be able to help improve the frequency and timing of ovulation, determine zygote quality and promote the ideal hormonal secretions for optimum fertility. The results of studies indicate that low levels of various nutrients often correlates with lower in vitro fertilisation (IVF) success rates, So that Melatonin has been demonstrated in multiple studies to improve zygote quality and almost double IVF success rates when taken for 1-3 months at a dose of 3 mg at night prior to IVF. Alpha-Lipolic Acid is another nutrient which has been demonstrated to be helpful to women with Polycystic ovary syndrome (PCOS) when taken as a twice daily controlled release 600 mg tablet. Iron has been shown to reduce the incidence of ovulatory infertility by 50% and is a very common deficiency. Vitamin D has been shown to have a powerful effect on increasing the odds of IVF success and has been shown to be important for a healthy pregnancy too. Vitamin C - in small doses of 750 mg daily has been shown to correct luteal-phase-defect which is marked by low progesterone in the second half of the menstrual cycle. This treatment boosted progesterone and estrogen levels and resulted in a 25% pregnancy rate within six months in a study published in Fertility and Sterility. Fish oil is a powerful anti-inflammatory which may help curb conditions such as endometriosis and has been demonstrated to increase uterine blood flow. Other vitamins that affect on fertility and should be consumed daily include vitamin E 400-800 units, Zn 15-60 mg, selenium 100-200 micrograms.

Conclusion: Vitamins and minerals are powerful regulators of fertility and numerous studies have pinpointed specific nutrients which may help women to up the odds of conception. Complementary medicine with fertility enhancement can be used as alternatives or complementary to chemical drugs in which they affect fertility.

Keywords: Infertility, Complementary Medicine, In Vitro Fertilization

P_{nm}-12: The Study of the Relationship between Lifestyle and Endometriosis in Infertile Women

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Background: Endometriosis is a chronic and prevalent disease in the world in which endometrium and endometrial stroma are found outside the uterus cavity. The presence of this tissue in various parts of the body causes various symptoms that have an adverse effect on women's quality of life, fertility and productivity. In this study, we reviewed the relationship between lifestyle and endometriosis.

Materials and Methods: This study is an analytical study and case-control. Sampling method In this study, easy sampling was performed in all infertility women undergoing laparoscopy, case group (with endometriosis) and control group (without endometriosis) in this study. The sample size according to the Cochran formula has been estimated in each group of 125 cases. The data collection tool is a demographic questionnaire including sex, body mass index, education, menstruation discipline and occupational status (type of shift). And the Standard lifestyle questionnaire is questionable in three areas: nutrition, activity, and smoking. Data were analyzed by SPSS software version 16. Quantitative variables were used by t-test and chi-square test was used to compare qualitative variables.

Results: There was a direct and significant relationship between the probability of endometriosis and the high age, education, and regularity of menstruation, so that with the increase of each of the above variables, the chance of developing the disease increases. Also, there was a significant and inverse association between endometriosis and body mass index, which means that with the increase of this variable, the risk of endometriosis in individuals decreases. No significant relationship was found between endometriosis and other variables. These variables include: Occupation and lifestyle in the three areas of activity - health, nutrition and smoking.

Conclusion: Our study suggests that high age, low body mass index, menstrual discipline had a significant relationship with endometriosis in infertile women and any relationship between lifestyle and endometriosis was not observed in infertile women.

Keywords: Infertility, Endometriosis, Lifestyle, Laparoscopy

P_{nm}-13: Sexual Dysfunction in Infertile Women: A Systematic Review

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Background: Sexual health is an important aspect of life. Sexual dysfunction is a common problem affecting women (40%) more than men (20% to 30%). Suggested factors that affect function in females are pregnancy, breast feeding, childbirth, interpersonal relationship with partner, socioeconomic factors like poor educational, level and low income, past history of sexual abuse, circumcision, medical disorders as diabetes, certain medications like hormonal contraceptives. Infertility is a common problem affecting 12/5 % of couples all over the world. Infertility has negative impact on physical and emotional health. Present study was conducted to evaluate the impact of infertile-

ity on the sexual function in infertile women.

Materials and Methods: Searching for information sources (SID, Science Direct, Google Scholar, SCOPUS, PubMed) was carried out on conducted studies in the past decade. Words Female sexual function index, Sexual disorder, Infertility, Women were used as key words and after removal of repetitive and common articles of data bases, 25 articles were obtained. Subsequently, by a closer look at the titles and objects of them, the results of 14 related articles were extracted.

Results: Reviewed articles showed based on the comparative studies, sexual dysfunction prevalence was higher in women with infertility. Sexual dysfunction prevalence in infertile women varied from 35/5 to 87 %.

Conclusion: Infertility was associated with an increase in female sexual dysfunction. The most affected areas of sexual function were lubrication, orgasm, and satisfaction. So infertility should be carefully considered during sexual consultation.

Keywords: Infertility, Sexual Disorder, Female Sexual Function Index, Women

P_{nm}-14: Preconception Lifestyle Advice for People with Subfertility in Iran

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Background: Infertility is a prevalent problem and has significant consequences for individuals and families. Several studies have shown the relationship between infertility and lifestyle. It is important to determine what preconception advice should be given about these types of factors to such people in order to help them to make positive changes and improve their chances of conception and delivering a healthy, live baby. Present study was conducted the effects of preconception advice on the chances of a live birth for people who perceive that they may be infertile and are investigating the possibility of medical treatment to address subfertility in Iran.

Materials and Methods: Searching for information sources (SID, Science Direct, Google Scholar, SCOPUS, PubMed) was carried out on conducted studies in the past decade. Words infertility, lifestyle, Iran and preconception advice were used as key words and after removal of repetitive and common articles of data bases, 34 articles were obtained. Subsequently, by a closer look at the titles and objects of them, the results of 16 related articles were extracted.

Results: The most frequently observed risk factors for infertility was smoking, body mass index (BMI) lower than 18.5 kg/m² and higher than 25 kg/m², over-exercising or not exercising at all, alcohol consumption, caffeine consumption of more than 300 mg/day, and stress. Although evidence has been found of the positive effects of a health-promoting lifestyle on infertility, no randomized controlled studies were found in literature which analyzed the effect of these risk factors on the success of assisted reproduction treatment in cases of unexplained infertility.

Conclusion: This review found no evidence from controlled clinical trials about the effect of preconception advice on the chance of a live birth in subfertile people. Moreover, there is no current guideline about what preconception advice should be

offered to people presenting for infertility treatment.

Keywords: Infertility, Lifestyle, Iran, Preconception Advice

P_{nm}-15: Micronutrient Supplementation and IVF Outcomes: What is its Effect?

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Background: Despite significant technological advances and developments in the treatment of infertility by IVF, only about 29% of treatments have resulted in live births after the first complete cycle. Several lifestyle-related factors have been shown to negatively impact the outcomes for patients undergoing IVF, specifically higher body mass index (BMI) and nutrition, including micronutrient deficiencies. There is much evidence on the importance of micronutrients in improving fertility in couples undergoing IVF therapy. Despite this, studies reporting the relevant clinical outcomes of IVF, such as pregnancy and live birth rates, are very scarce. The aim of this review is to summarize clinical evidence on the effect of micronutrients on primary outcome parameters of IVF treatment.

Materials and Methods: This review is written with an overview of related articles in Scencedirect, Pubmed, cochrane and Embase for articles from the inception of each database to April 2018.

Results: The studies were spread across the world with one study each from Australia, Egypt, France and Iran. The interventions were highly heterogeneous among the included studies. From 7 studies that include in this review four studies reported fertilization rates ranging from 29 to 73% for intervention and 19 to 71% for controls. Three other studies reported a higher fertilization rate in the intervention group. Pregnancy rates were higher in the intervention groups in all the Randomized clinical trials (RCTs). Other outcomes include higher implantation rate and live birth rates in intervention groups.

Conclusion: Micronutrients appear to influence positive outcomes in couples undergoing fertility treatment. Vitamin and antioxidant supplementation improves semen quality but women tend to show varying outcomes. Larger clinical studies are needed to strengthen these findings so that the benefit of micronutrients can be extended to subjects undergoing IVF therapy.

Keywords: Infertility, Micronutrients, IVF, Outcome

P_{nm}-16: A Commercialized Look at The Assisted Reproductive Services Provided by a Third Party

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Background: In the new millennium, the assisted reproductive technology has become a global business, and international markets of human gamete/embryo is considered a global health problem. Increased desire of people to use modern assisted reproductive technology on the one hand and the limitations of gamete/embryo on the other hand has led human cells to be traded as a favorable product in international markets. Undoubtedly, the commercialization of reproduction is an issue which requires ethical considerations and necessary solutions.

Materials and Methods: The present study is a review article carried out based on searching the keywords of "Infertility", "Assisted Reproductive Technologies", "Gamete Donation", "Embryo Donation", "Third Party Reproduction", "Medical Ethics" and "Medical Rights" from 5 databases including PubMed, Web of Science, Scopus, Cochrane, EM Base and Med line.

Results: Studies on the ethical and legal aspects of the gamete/embryo donation in different countries throughout the world attribute this problem to economic gaps, differences in the laws and policies of countries regarding the application of these technologies, weakness of the regulatory system, ignoring ethical dimensions of the subject, and not including them in the existing laws. All of which result in the phenomenon of reproductive tourism, looking at human gamete/embryo as a good, substitution of donation with business, doubts about the validity of assistive reproductive contracts with the participation of third parties, instrumental use of individuals for artificial reproduction, and conversion of service providers to market-like organizations.

Conclusion: Addressing the issues related to financial aspects and commercial outcomes of gamete/embryo donation reveal that the use of such methods, without recognizing its various aspects and creating suitable substrates for preventing any misuse, has changed this assistive reproductive method, which has realized the dream of many infertile couples on childbearing, to a global crisis. Undoubtedly, these outcomes are not favored by the first therapists or policymakers in the field of infertility treatment, but they are rather inadvertently due to the combination of specific conditions. In this regard, the following strategies can be suggested; controlling misuses through regulations, planning, and legal definition of financial relations between donor/receiver of gamete/embryo, the use of insurance services for a fair distribution of low-cost health facilities, a comprehensive and coordinate international response, coordination of domestic laws of the countries, and developing solutions for international management of this global problem.

Keywords: Medical Ethics, Medical Rights, Third Party Reproduction, Gamete Donation, Embryo Donation

P_{nm}-17: Justice in Provision of Assisted Reproductive Services and its Conflicts with The Rights of Children Born Through Gamete/Embryo Donation

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Background: Establishing social justice in the system of health services and the fair allocation of financial and non-financial resources among the general public is a main ethical issue. This subject is addressed in both prioritizing the resources at the community level and rationing and prioritizing individuals for services. The realization of justice, especially in the field of health, is affected by many factors; therefore, establishment of justice, even willing to realize it, is difficult in today's complex societies. One of these difficulties is the confliction of the rights of applicants of this assisted reproductive method and the benefits of children born through this method, which occurs due to a legal defect in the manner of embryo donation and ensuring the benefits of children born through this method.

Materials and Methods: The present study is a review article carried out based on searching the keywords of "Infertility", "Assisted Reproductive Technologies", "Gamete Donation", "Embryo Donation", "Medical Ethics", "Medical Rights" and "Justice" from 5 databases including PubMed, Web of Science, Scopus, Cochrane, EM Base and Med line.

Results: The most important examples of the justice principle in the issue of gamete/embryo donation are the need for everyone to have a fair access to these assisted reproductive services and to respect the reproduction freedom right of applicants receiving gamete/embryo. A study for evaluation of infertility centers in Tehran in 2014-2015 confirmed the provision of these services to applicants without any specific restrictions or accurate screening.

Conclusion: Fertility is the natural right of humans; therefore, infertility treatment is also the right of individuals, but one person deserves to receive this right to the extent that it is not inconsistent with the rights of another person, such as the born child. Given the lack of guarantees of the benefits of children born through donation in the law on the method of embryo donation, as well as the low-level of social support of children in Iran, unlimited provision of assisted reproductive services for donation of gamete/embryo to the applicants is subject to adoption of laws that could eliminate or reduce further concerns arising from the use of this technology. Until realization of this issue, screening of applicants to identify eligible people requires ethical decisions.

Keywords: Medical Ethics, Medical Rights, Gamete Donation, Embryo Donation, Justice

P_{nm}-18: Sex Selection: An Ethical Issue or An Absolute Right? A Review Article

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Background: This review article investigates the moral and ethical aspects of sex selection; with an overview of negative and positive ideas holding around this global issue.

Materials and Methods: A series of searches was conducted of Medline databases published in English between 1996 and 2018.

Results: While abortion or infanticide has long been used as

means of sex selection, a new technology preimplantation genetic diagnosis (PGD) has become a highly efficient, and arguably less controversial, way of ensuring the birth of a child of a particular sex. Sex-selection via genetic testing has had mixed reactions from the public, and has resulted in a great deal of criticism from people who argue that the practice promotes gender discrimination and could lead to gender imbalance in the population. The central argument put forward is that non-medical sex selection is a practice which promotes socially restrictive conceptions of sex, gender and family. Those against it have argued that sex selection is akin to “playing God”, by interfering with the natural process of reproduction. Non-medical sex selection is often seen as problematic in countries that have a son preference but not in Western countries which appear to use sex selection for “gender-balancing”. Advocates of sex selection use a rights-based liberal approach as the framework for evaluating reproductive technologies in general and PGD for sex selection in particular. Under this framework, reproductive choice and parental autonomy are basic freedoms. Interference with individuals’ autonomous reproductive and parental choices is illegitimate, unless their actions can be shown to clearly and seriously harm others.

Conclusion: Sex selection is still an advocated practice among people of some countries because of special ideas related to their culture. People also use sex selection for family balancing. However this practice causes some global or local issues in today’s societies, sex selection is still a controversial issue.

Keywords: Ethical Issues, Morality, Sex Selection, Review Article, Genetic Testing

P_{nm}-19: The Evaluation of The Relationship between Some Related Hormone Levels and Diet in Obese or Overweight Patients with Hirsutism: A Randomized Clinical Trial

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Background: Hirsutism is a common disorder that has remarkable physical and mental effects on individuals. No appropriate diet has yet specified for individuals with hirsutism. The present study was carried out to examine the effect of high fibre, low caloric balanced diet on some related hormone levels in obese or overweight women with hirsutism who had referred to clinics affiliated with Shiraz University of Medical Sciences.

Materials and Methods: The present study was a clinical trial that was carried out on 47 obese or overweight women with hirsutism in 2014. The women were randomly assigned to an intervention group and a control group that, respectively, consumed a high fiber, low caloric balanced diet and a nor-

mal diet for 3 months. A demographic characteristics questionnaire and a researcher designed diet questionnaire were filled out by the two groups before the intervention. Before and 12 weeks after the intervention, body mass index (BMI) was measured and blood samples (on the 3-5 days of menstruation) were collected. Factors of luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, dehydroepiandrosterone sulfate, low-density lipoproteins, high-density lipoproteins (HDL), fasting blood sugar (FBS), CHOL, prolactin, triglycerides, insulin, 17 hydroxyprogesterone, and free androstenedione testosterone were measured. The collected data were analyzed through t test, Chi square, and intergroup analysis using SPSS 22.0.

Results: The mean age of the participating women was 27.23 ± 5.42 years. After the study, the level of FBS and insulin in the intervention group dropped while they increased in the control group. Moreover, the postintervention level of BMI in the intervention group on average decreased 1.89 units while it rose by 0.3 units in the control group, and there was a significant difference between the two groups (P<0.001).

Conclusion: The results of the present study showed that consuming high fiber diet by obese or overweight women with hirsutism and polycystic ovary can reduce some factors including the level of FBS, insulin, and cholesterol and enhance blood HDL. Therefore, consuming this type of diet is recommended to treat this disorder.

Keywords: High Fiber Diet, Hirsutism, Obesity, Overweight, Sex Hormones

P_{nm}-20: Effect of Vitamin C Supplementation on The Levels of Related Hormones in Infertile Women with Polycystic Ovary Syndrome in Shiraz City

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Background: Infertility is one of the most problems in the world today. Polycystic ovary syndrome (PCOS) is a common cause of infertility in women. Vitamin C as a single chain antioxidant can stop the release of peroxidation processes in the body and increase of progesterone level as well as increase the effect of Clomiphene on ovary. This study aimed to determine the effectiveness of vitamin c in a balanced diet on the levels of related hormones in infertile women with Hay-polycystic ovary syndrome in Shiraz city.

Materials and Methods: This study was a randomized clinical

trial (RCT), double-blind cross-over between 2012-2013 infertile women with PCO were performed on 56 randomly into two groups, A, B, were carried out. Screening samples from infertile patients with PCO groups with the criterion of three criteria: the Rotterdam study and experiments, FSH, Testosterone, Progesterone, Estradiol primarily carried out and then the course of 2 months of placebo (B) and Vitamin C (A) the tests were given 3 times a month before and 2 months after the first intervention was performed and the results were evaluated in both groups.

Results: In the group treated with vitamin A and C in the first stage of the hormones FSH, estradiol, and more Testosterone treatment group B increased, but not statistically significant. Also, the effect of treatment with hormones FSH, Testosterone, Progesterone, and Estradiol was found that the amount of drug effect is not significant.

Conclusion: The findings of this study showed that vitamin C can cause changes in the levels of certain hormones, but hormones have no effect on the comparison of the mean scores.

Keywords: Vitamin C, Related Hormones in Women, Infertility, Polycystic Ovaries Syndrome, Balanced Diet

Can Yoga Exercises Improve In Vitro Fertilization Outcomes in Women with Polycystic Ovary Syndrome?

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Background: Polycystic ovary syndrome (PCOS) is considered as the most common cause of Infertility. Yoga exercises can be noticed as an adjuvant therapy in infertile women with PCOS. This study aimed to determine The Effect of Yoga on In vitro fertilization Outcomes in Women with Polycystic Ovary Syndrome Undergoing Infertility Treatment.

Materials and Methods: This clinical trial was carried out on 61 infertile women with PCOS (31 women in case and 30 in control group) undergoing infertility treatment referred to the Sarem Hospital in Tehran. Demographic, fertility, and data about IVF outcomes were collected using researcher made questionnaires before and after 6 weeks yoga in two groups. The collected data were analyzed using SPSS 21 software.

Results: The mean age of women was 30.77 ±6.01 and 30.35 ± 5.53 years in case and control groups respectively (p=0.259). The most women in two groups (56.7% vs. 61.3% in case and control ones respectively) had academic education (p=0.358). There were not any significant differences between 2 groups in terms of follicle numbers after ovarian stimulation, number of embryos after egg fertilization, and pregnancy occurrence detected by B-HCG positive test 14 days after embryo transfer.

Conclusion: Six weeks yoga exercises didn't show any significant effect on in vitro fertilization outcomes in women with PCOS undergoing infertility treatment.

Keywords: Infertility, Poly Cystic Ovary Syndrome, Yoga, In Vitro Fertilization

Evaluation of changes in screening tests in the first trimester of fertility in women with polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases among women in reproductive ages. Its prevalence in various studies is 5-10%. Serum levels of both β -HCG and PAPP-A are closely related to decaying and pairing processes. Arterial vascular changes as a result of endothelial damage in pregnant women without PCOS are reported in several reports. In addition to endothelial damage, the disorder of the decidua trophoblast attack is seen in endometrial spiral arteries and muscle, which is related to the failure of the pairs. It can be concluded that these changes are seen in patients with PCOS.

Methods: This study is a review study after searching in databases such as Proquest, Scopus, Springer and Science Direct PubMed, google scholar with keywords such as PCOS and first trimester screening tests and pregnancy. 10 full text articles published between 2004 and 2016 was obtained.

Results: In studies, high levels of BHCG have been seen in the group with pcos compared with the control group, while in other studies, PAPP-A and BHCG in the PCOS group were significantly lower than those in the control group But in all studies, there was no difference between the two groups in terms of NT.

Discussion: Generally, the level of biochemical compounds in the first trimester of pregnancy has been altered in women with PCOS, but there is a need for more extensive studies in this regard. Since the above study did not have the same, successful internal and external study, currently, screening for these patients is not recommended, so further studies are recommended.

Key words: Polycystic ovary syndrome, first trimester screening tests and pregnancy.

Survey the Relationship of Infertility-Related Stress with Spiritual Health in Infertile Women Referred to Shahid Mottahari Treatment and Educational Center, Urmia, 2017

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Background: One of the problems of mental health is infertility that has a stressful impact on both partners, with adverse effects on mental health of infertile women. Spirituality is a positive, meaning-based strategy that can help infertile women coping with infertility stress, protecting them against its complications. No study has investigated the relationship of infertility-related stress with spiritual health in infertile women.

Materials and Methods: The present study has employed correlational methodology. The study population comprised 400 infertile women referred to Shahid Mottahari treatment and educational center, Urmia, Between January and December 2017, 400 infertile women starting their first fertility treatment at Shahid Mottahari treatment and educational center, Urmia, Iran were recruited. The study sampling was purposeful. As-

assessment tools used in this study were as follows: A self-reporting questionnaire, containing the (Spiritual Well-Being Scale; SWBS), by Paloutzian & Ellison, Newton infertility stress questionnaire, and demographic questionnaire. Participants completed self-report of spirituality, infertility-related stress, and their demographic characteristics. For statistical analysis, we used test correlation coefficients of data, multiple regression analysis, and logistic regression.

Results: There was a meaningful significance of women's spirituality wellbeing on their own their infertility-related stress (0.043). Moreover, the direct effect of age and level of economic situation on their spirituality wellbeing was significant ($P= 0.17, 0.06$). There were not significant effects of marriage duration and educational level on women's spirituality as well as on their infertility-related-stress ($P= 0.22, 0.75$).

Conclusion: Findings highlight the importance of a baseline assessment of spirituality as a personal resource that infertile women might use to overcome the aversive effects of infertility. Nursing interventions aimed to encourage spirituality have the potential to promote treatment by decreasing infertility related stress in the clients.

Keywords: Infertility, Stress, Spiritual Health, Infertile, Women

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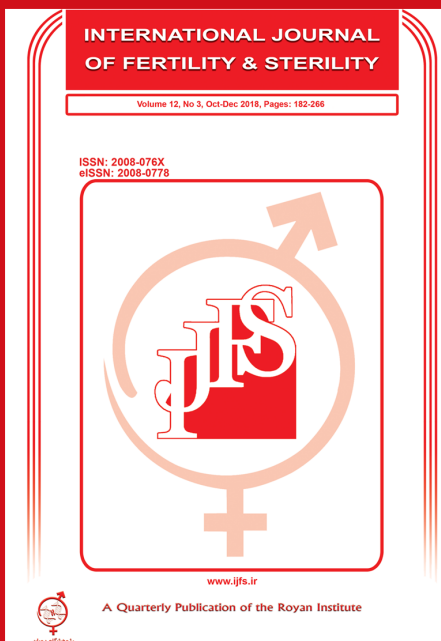
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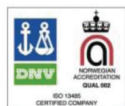
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