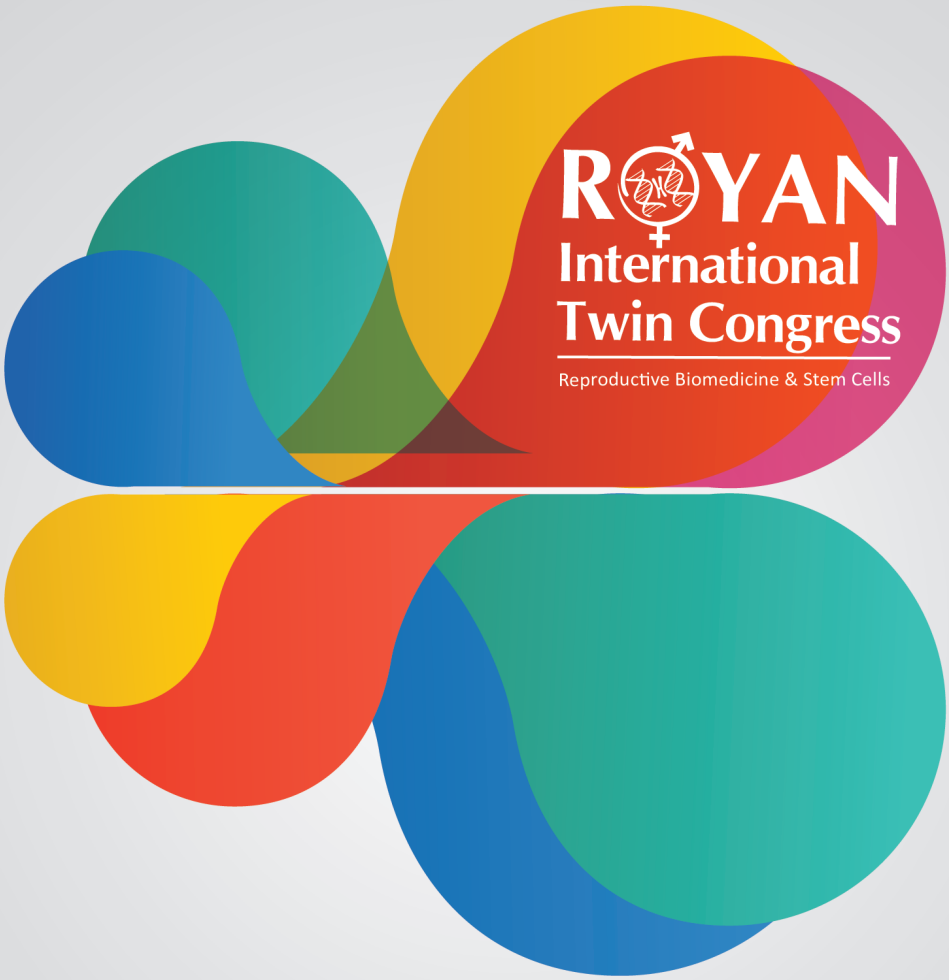
ROYAN

International Twin Congress

August 30 - September 1 , 2017 - Tehran

Milad Tower International Conference Hall

ABSTRACT BOOK



18th Congress on Reproductive Biomedicine

13th Congress on Stem Cell Biology & Technology

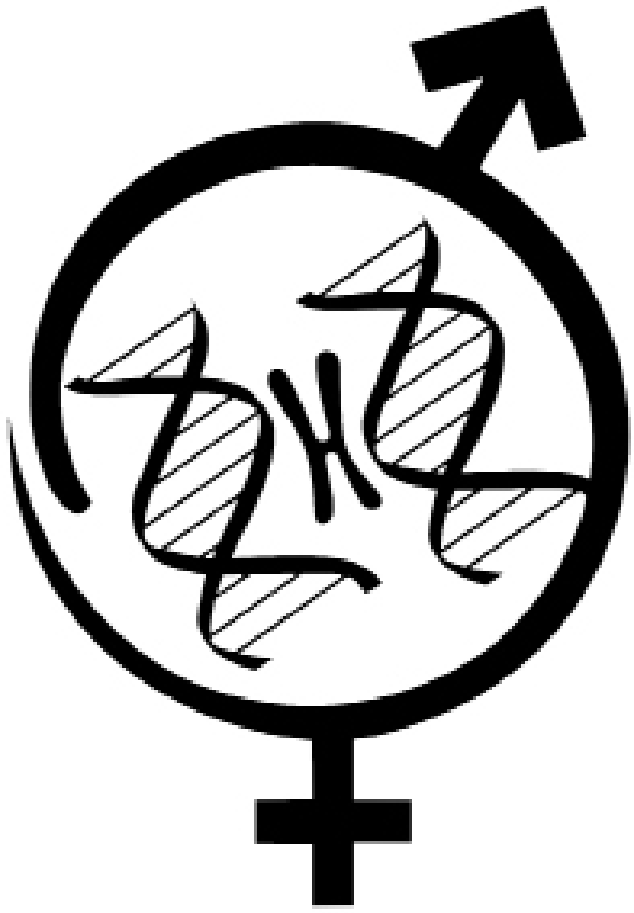
www.royancongress.com

Abstracts of

Royan International Twin Congress

13th Congress on Stem Cell Biology and Technology

30 August - 1 September 2017



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Abstracts of the 13th Congress on Stem Cell Biology and Technology (2017)

Contents:

Congress President:
Gourabi H., Ph.D.

Congress Chairman:
Shahpasand K., Ph.D.

Committees
Organizing Committee:
Abdollahian E., B.Sc
Afaharian P., Ph.D
Ahmadi SE., M.Sc
Alizadeh SK., B.Sc
Azimi R., M.Sc
Bahrvand H., Ph.D
Daliri L., M.Sc
Ezabadi Z., M.Sc
Farrokh S., B.Sc
Fathi R., Ph.D
Irani Sh., Ph.D
Lotfipanah M., M.Sc
Kashfi F., M.Sc
Shahverdi AH., Ph.D
Shahzadeh Fazeli A., Ph.D
Tavassolian R., B.Sc
Vosough M, MD., Ph.D
Vosough Taghi Dizaj A., M.D.
Zarrabi M., M.D

Executive Committee:
Amirchaghmaghi E.
Abdi F.
Dadkhah F.
Ghadami F.
Ghodsi A.
Kouhkan A.
Malekzadeh F.
Mesami F.
Mirshekar Z.
Pahlavan S.
Roohbani Sh.
Sheikhan M.
Taheri E.
Vasefi N.
Zoghi F.

Scientific Board

Abroun S.
Aghdami N.
Ali Moghaddam K.
Al-Jamal KT.
Araúzo-Bravo MJ.
Atari M.
Baghban Eslami Nejad M.
Baharvand H.
Bahrami AR.
Bober E.
Braun T.
Didar T.
Dominici M.
Ebrahimi M.
El-Shanti H.
Farzaneh F.
Fathi A.
Gee AP.
Ghaedi K.
Gheraati MR.
Grapin-Botton A.
Hamidieh A.A.
Hanley PJ.
Hassani N.
Heydari S.
Hisanaga SI.
Hosseini Salekdeh GH.
Karaoz E.
Khademhosseini A.
Khosrotehrani K.
Kiani S.
Larijani B.
Lipp P.
Malekzadeh R.
Moghaddam MM.
Moghaddasali R.
Mozafari S.
Niknejad H.
Nagy A.
Najimi M.
Olsson MJ.
Samadi Kuchaksaraei A.
Sanati Nezhad A.
Pahlavan S.
Rouzafzoon R.
Shahpasand K.
Shahzadeh Fazeli SA.
Shekari F.
Soleimani M.
Tahamtani Y.
Totonchi M.
Vosough M.
Walter J.

Collaborators in Award Jury and Scientific Board (Stem Cell)-2017

Royan Institute, Iran
Iranian Stem Cell Network
Iranian Council of Stem Cell Technology
(ICST) AO Foundation, Switzerland
- **AO Research Institute Davos (ARI)**

Cancer Reseach Institute, Slovakia
Eberhard Karls University Tübingen, Germany
- Department of Traumatology Schnarrenbergstr

Isfahan Medical University, Iran
- Department of Genetic and Molecular Biology

Eurocord Hopital, France
Ferdowsi University of Mashhad, Iran
- Department of Biology

Fraunhofer Institute for Biomedical Engineering IBMT, Germany
French Institute of Health and Medical Research, France
Hannover Medical School, Germany
- Department of Stem Cells Biology

ICM Institute for Brain and Spinal Cord, France
Institute for Neurophysiology, Germany
Institute of Biomedicine of Valencia, Spain
Italian National Research Council, Italy
- Institute of Genetics and Biophysics, Adriano Buzzati Traverso (IGB) Kangwon National University, South Korea
- Department of Electronics Engineering

Karolinska University Hospital, Sweden
- Department of Laboratory Medicine, Division of Pathology

Keio University, Japan
- Department of Internal Medicine

Kurdistan University of Medical Sciences, Sanandaj, Iran
- Department of Anatomy, School of Medicine

Laboratory of Stem Cell Bioengineering LSCB, Switzerland
Mario Negri Institute for Pharmacological Research, Italy
- Department of Molecular Medicine

Murdoch Children's Research Institute, Australia
- Royal Children's Hospital

Nagoya City University, Japan
Nanyang Technological University, Singapore
National University of Ireland, Galway
- Centre for Research in Medical Devices (CÚRAM)

National Institutes of Health, USA
- National Center for Advancing Translational Sciences, Division of Pre-Clinical Innovation National Institute of Immunology, India

Pasteur Institute of Iran (IPI), Tehran, Iran
- National Cell Bank of Iran

Peking University, China
- Department of Cell Biology, School of Basic Medical Sciences, Stem Cell Research Center San Raffaele Scientific Institute, Italy
- Division of Neuroscience, Institute of Experimental Neurology (INSPe)

- Diabetes Research Institute (HSR-DRI)
- Program in Immunology and Bio-immunotherapy of Cancer PIBIC
- Complications of Diabetes Unit

Taiyuan University of Technology, China

- Department of Chemistry

Tajen University, Taiwan

- College of Health Sciences

Tarbiat Modares University, Iran

- Department of Hematology, Faculty of Medical Science
- Department of Anatomy, Faculty of Medical Science
- Department of Molecular Genetics

Tehran University of Medical Sciences, Iran

- Hematology, Oncology and Stem Cell Transplantation Research Center, Shariati Hospital The Rockefeller University, USA
- Laboratory of Stem Cell Biology and Molecular Embryology

TWICORE, Centre for Experimental and Clinical Infection Research, Germany

University Hospital Halle (Saale), Germany

- Department of Hematology/Oncology

University of Cincinnati, College of Medicine, USA

- Department of Molecular Genetics

University of Cologne, Germany

- Institute for Neurophysiology

University of Coimbra, Portugal

- Center for Neurosciences and Cell Biology

University of California, USA

University of Manitoba, Canada

University of Strasbourg, France

Congress Chairman

Koorosh Shahpasand

Dear Colleagues,

It gives me the greatest pleasure to invite you to join us at **13th International Congress on Stem Cell Biology** and Technology to be held in Tehran in September 2017. We have been featuring latest findings in this field since 2005 and we hope to follow in the successful footsteps of the meetings in 2017.

The program committee is planning a fabulous program ranging across the spectrum of stem cell science. The scientific exchange quality in previous meetings and increasing number of participants has made the congress a leading stem cell research and instruc-tional meeting in the region. We will have 18th International congress on Reproductive Biomedicine in parallel to this event, which will be held by Royan Institute.

Royan institute was established in 1991 by the late Dr. Kazemi Ashtiani which is one of the most pioneering institutes conducting basic and translational research on stem cells, developmental biology and regenerative medicine. The members of the local organizing committee are very proud to be hosting the ICSCBT2017 and look forward to welcom-ing you to our city and country. Tehran and its environs are an amazing place to visit. Our multicultural city has many diverse neighborhoods of interest to visitors. The meet-ing promises to be highly rewarding on a social basis as well as on an intellectual basis. At the end of this Congress, we hope that attendees feel that they have garnered the most up-to-date information available in Developmental Biology. Please mark in your calendars the dates of the ICSCBT2017, September 2017, so that you may join your colleagues in what we hope will be a fantastic meeting.

Koorosh Shahpasand, Ph.D.
Congress Chairman of 13th Congress
on Stem Cell Biology and Technology

Invited Speakers

Is-1: Computational Epigenomics Tools to Understand The Cellular Language of Pluripotency and Reprogramming

Araúzo-Bravo MJ

Biodonostia Health Research Institute, Computational Biology and Systems Biomedicine, Calle Doctor Beguiristain, San Sebastián, Spain
Email: mararabra@yahoo.co.uk

Cellular reprogramming is key technology in regenerative medicine. The reprogramming process is based on the crosstalk between genetic and epigenetic networks in a language whose words are DNA regulatory sequences. To interpret such language we designed computational tools that discover "DNA words" with genetic and epigenetic meaning. We developed software to search of abinitio DNA patterns, to process efficiently DNA methylomics data, and to predict superenhancers from next generation sequencing epigenomics data.
Our computational method to discover "DNA words" with regulatory meaning exploits never used transcription factors (TFs) properties to reveal the missing TF binding motives (TFBMs) and their sites (TFBSs) in all human gene promoters. We disclose the crosstalk between "DNA words" with an algorithm that extracts TF combinatorial binding patterns compiling a collection of TF regulatory syntactic rules. Our TF binding site map for combinatory TFBMs discovery provides a comprehensive resource for regulation analysis that includes a dictionary of ''DNA words, '' newly predicted motifs and their corresponding combinatorial patterns that represent syntax of gene regulation. Compiling the epigenomic counterpart of the dictionary of TFBMs requires processing of massive quantities of Bisulfite sequencing (BSseq) data. We developed P3BSseq, a parallel processing pipeline for fast, accurate and automatic analysis of BSseq reads that trims, aligns, annotates, records the intermediate results, performs bisulfite conversion quality assessment, generates BED methylome and report files following the NIH standards.
Gene expression regulation is gated by DNA promoter methylation states modulating TF binding. The known DNA methylation/unmethylation mechanisms are sequence unspecific, but different cells with the same genome have different methylomes, thus additional processes bringing specificity to the methylation/unmethylation mechanisms are required. Searching for such processes, we demonstrated that CpG methylation states are influenced by the sequence context surrounding the CpGs. We used such a property to develop a CpG methylation motif discovery algorithm. The discovered motifs reveal ''methylation/unmethylation factors'' that could recruit the ''methylation/unmethylation machinery'' to the loci specified by the motifs. The motifs that were found discriminate between hypomethylated and hypermethylated regions and represent a dictionary of "DNA methylation words".
Other longer length DNA words are the superenhancers (SE), structural genomic elements determining cell fate and considered epigenetic syntactic elements. We developed NaviSE for fullyautomated parallel processing of genomewide epigenomics data. NaviSE implements "epigenomics signal algebra" that allows the combination of multiple activation and repression epigenomics signals.
NaviSE annotates the SE associated genes and performs gene ontology enrichment analysis, TFBSs enriched in SE, protein protein interaction networks and enriched metabolic pathways, giving meaning to the "DNA words" of the gene regulation language.

Is-2: Computational Biology Analysis of Transcriptomics Dynamics Identifies Genes Specific to Primordial Germ Cells

Araúzo-Bravo MJ

Biodonostia Health Research Institute, Computational Biology and Systems Biomedicine, Calle Doctor Beguiristain, San Sebastián, Spain
Email: mararabra@yahoo.co.uk

The molecular mechanisms underlying PGC specification are poorly understood due to inaccessibility of cell material and lack of *in vitro* models for tracking the earliest stages of germ cell development. PGC specification in mouse starts at E6.0–6.5 post implantation. We designed a singlecell transcriptomics dynamics computational study to identify markers of the PE–EPI bifurcation in ICM cells through searching for statistically significant differently expressed genes (DEGs) between PE and EPI cells from E3.5 to E4.5. The DEGs common for E3.5 and E4.5 were used as the markers defining the steady states. We collected microarray and next generation sequencing transcriptomics data from public databases from bulk populations and single cells from mice at E3.25, E3.5 and E4.5. We identified a collection of previously undescribed E3.5 specific PE and EPI markers, and new steady PE and EPI markers. We found that mouse PGC marker activation starts at least at E3.25 preimplantation. Since it is so well established in the literature that mouse PGC specification is a postimplantation event, it was surprising to see activation of PGC markers as early as E3.25 preimplantation, and identify the newly found steady EPI markers as late germ cell markers. The early activation of PGC markers points out the difficulty of separating PGC cells from pluripotent populations. Our results suggested that the combining of the precision of single cell omics data with dynamic analysis of time series data can establish the timing of some developmental stages as earlier than previously thought. Deciphering the crosstalk between transcription factors and DNA methyltransferases is important to understand early PGC development. TCFAP2C has a CpG DNA methylation motif not methylated in pluripotent cells and that could potentially bind on DNMT3L. The transcriptomics dynamics analyses support the regulation of Dnmt3l expression by TCFAP2C.
At mouse embyo postimplantation only a few genes that mark the onset of germ cell commitment in the epiblast including tissue nonspecific alkaline phosphatase, Blimp1, Stella and Fragilis have been used with some success to detect PGC formation in *in vitro* model systems. We identified 11 genes (three of which are novel) that are specifically expressed in male and female fetal germ cells, both *in vivo* and *in vitro*, but are not expressed in ESCs. Expression of these genes allows us to distinguish committed germ cells from undifferentiated pluripotent

cell populations, a prerequisite for the successful derivation of germ cells and gametes *in vitro*.
In the human case we found that in response to cytokines, PSCs differentiate first into a heterogeneous mesoderm like cell population and then into PGC like cells with minimal PRDM14 expression. PGC specification in humans is similar to mouse, with the sequential activation of mesodermal and PGC genes, and the suppression of neural induction and of de novo DNA methylation. Using an interspecies transcriptomics approach we found that PGC commitment in humans has key differences from mouse, including transcriptional regulation during the early stage PGC development.

Is-3: Dental Pulp Pluripotent Like Stem Cells (DPPSC), A New Stem Cell Population with Chromosomal Stability and Osteogenic Capacity for Biomaterials Evaluation

Atari M

Department of Medical Surgical Pathology, UIC Regenerative Medicine Research Institute, International University of Catalunya, Barcelona, Spain
Email: matari@uic.es

Background: Biomaterials are widely used to regenerate or substitute bone tissue. In order to evaluate their potential use for clinical applications, these need to be tested and evaluated *in vitro* with cell culture models. Frequently, immortalized osteoblastic cell lines are used in these studies. However, their uncontrolled proliferation rate, phenotypic changes or aberrations in mitotic processes limits their use in longterm investigations. Recently, we described a new pluripotent like subpopulation of dental pulp stem cells derived from the third molars (DPPSC) that shows genetic stability and shares some pluripotent characteristics with embryonic stem cells. In this study we aim to describe the use of DPPSC to test biomaterials, since we believe that the biomaterial cues will be more critical in order to enhance the differentiation of pluripotent stem cells.
Materials and Methods: The capacity of DPPSC to differentiate into osteogenic lineage was compared with human sarcoma osteogenic cell line (SAOS2). Collagen and titanium were used to assess the cell behavior in commonly used biomaterials. The analyses were performed by flow cytometry, alkaline phosphatase and mineralization stains, RT-PCR, immunohistochemistry, scanning electron microscopy, Western blot and enzymatic activity. Moreover, the genetic stability was evaluated and compared before and after differentiation by shortcomparative genomic hybridization (sCGH).
Results: DPPSC showed excellent differentiation into osteogenic lineages expressing bonerelated markers similar to SAOS2. When cells were cultured on biomaterials, DPPSC showed higher initial adhesion levels. Nevertheless, their osteogenic differentiation showed similar trend among both cell types. Interestingly, only DPPSC maintained a normal chromosomal dosage before and after differentiation on 2D monolayer and on biomaterials.
Conclusion: Taken together, these results promote the use of DPPSC as a new pluripotent-like cell model to evaluate the biocompatibility and the differentiation capacity of biomaterials used in bone regeneration.

Is-4: Dental Pulp of The Third Molar: A New Source of Pluripotent-Like Stem Cells

Atari M

Department of Medical Surgical Pathology, UIC Regenerative Medicine Research Institute, International University of Catalunya, Barcelona, Spain
Email: matari@uic.es

Background: Dental pulp is particularly interesting in regenerative medicine because of the accessibility and differentiation potential of the tissue. Dental pulp has an early developmental origin with multilineage differentiation potential as a result of its development during childhood and adolescence. However, no study has previously identified the presence of stem cell populations with embryonic-like phenotypes in human dental pulp from the third molar.
Methods and Results: In the present work, we describe a new population of dental pulp pluripotent-like stem cells (DPPSCs) that were isolated by culture in medium containing LIF, EGF and PDGF. These cells are SSEA4(+), OCT3/4(+), NANOG(+), SOX2(+), LIN28(+), CD13(+), CD105(+), CD34(-), CD45(-), CD90(+), CD29(+), CD73(+), STRO1(+) and CD146(-), and they show genetic stability *in vitro* based on genomic analysis with a newly described CGH technique. Interestingly, DPPSCs were able to form both embryoidbody-like structures (EBs) *in vitro* and teratoma-like structures that contained tissues derived from all three embryonic germ layers when injected in nude mice. We examined the capacity of DPPSCs to differentiate *in vitro* into tissues that have similar characteristics to mesoderm, endoderm and ectoderm layers in both 2D and 3D cultures. We performed a comparative RT-PCR analysis of GATA4, GATA6, MIXL1, NANOG, OCT3/4, SOX1 and SOX2 to determine the degree of similarity between DPPSCs, EBs and human induced pluripotent stem cells (hIPSCs).
Conclusion: Our analysis revealed that DPPSCs, hIPSC and EBs have the same gene expression profile. Because DPPSCs can be derived from healthy human molars from patients of different sexes and ages, they represent an easily accessible source of stem cells, which opens a range of new possibilities for regenerative medicine.

Is-5: Isolation and Expansion of Oogonial Stem Cells from Adult Mouse and Human Ovaries

Baharvand H

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, Cell Science Research Center, ACECR, Tehran, Iran
Email: baharvand@royaninstitute.org

Is-6: Novel Functions of Sirt7 in Securing Genomic Integrity during Cellular Stress Responses

Bober E

Department of Cardiac Development and Remodeling, Max Planck-Institute for Heart and Lung Research, Ludwigstrasse, Nauheim, Germany
Email: Eva.Bober@mpi-bn.mpg.de

Background: Sirtuins constitute a class of highly conserved

enzymes, which act preferentially as NAD+dependent deacetylases and/or mono ADP ribosyltransferases. Individual members of mammalian sirtuin family (Sirt1 to Sirt7) play decisive roles in different biological processes such as differentiation, proliferation, metabolism, and regulation of stress responses. We investigated the role of Sirt7 in cellular stress responses and in the maintenance of nucleolar architecture and genomic stability.
Materials and Methods: We used Sirt7 and Sirt1 knockout mice for *in vivo* studies and for generation of primary sirtuin deficient cells. In addition, a panel of cell lines with sirtuin knockdown or overexpression was used for *in vitro* experiments.
Results: Sirt7 is the only mammalian sirtuin, which is enriched in the nucleolus. We discovered that the nucleolar structure is impaired in Sirt7 deficient cells *in vitro* and *in vivo*. The underlying molecular mechanism involves loss of heterochromatin and loss of genomic repetitive sequences in rDNA and satellite DNA caused by lack of Sirt7. In wildtype cells Sirt7 recruits DNMT1 and Sirt1 to establish heterochromatin and ensure genomic stability. Beside general cellular functions, the most prominent role of Sirt7 is revealed during cellular stress. In response to UV irradiation Sirt7 contributes to p53 stabilization through the regulation of NPM Hdm2 p53 pathway. Furthermore, we discovered novel mechanism involving complex interrelation of Sirt7 with Sirt1 to inhibit rDNA transcription, prevent DNA damage and increase DNA repair capacity under stress conditions. Depending on the cellular context, however, Sirt7 may act not only as tumor suppressor but also as an oncogene.
Conclusions: Sirt7 regulates nucleolar stress through rDNA transcription inhibition dependent and independent mechanisms. Sirt7 and Sirt1 constitute a feedback loop mechanism to control rDNA inhibition in response to genotoxic stress.

Is-7: Compaction of Chromatin Seals Quiescence of Muscle Stem Cells

Braun T

Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
Email: Thomas.Braun@mpi-bn.mpg.de

Background: Skeletal muscle stem cells are indispensable for postnatal muscle growth, homeostasis and regeneration. Reduced activity or loss of muscle stem cells results in loss of skeletal muscle mass. Impaired muscle function has multiple adverse effects on the metabolism and restricts mobility often leading to lifethreatening conditions. Muscle stem cells persist mostly in a quiescent state, which is associated with a strong accumulation of transcriptionally silent heterochromatin. So far relatively little was known neither about the potential link between heterochromatin formation and MuSC quiescence nor about the genes regulating this state.
Materials and Methods: Immunofluorescence staining of chromatin marks and electron microscopy was used to determine the extent of heterochromatin formation in muscle stem cells and to characterize changes in histone tail modification during activation of muscle stem cells after induction of muscle regeneration. Targeted inactivation of the histone methyltransferase Suv420h1 in mice was applied to determine the effects of a loss of H4K20me2 modifications on muscle stem cell quiescence and muscle regeneration followed by generation of compound mouse mutants to investigate genetic interactions. Fluorescence in situ hybridization demonstrated repositioning of the MyoD gene locus during muscle stem cell activation. Chromatin immunoprecipitation was utilized to analyze heterochromatin formation at the MyoD gene locus. The effect of loss of stem cell quiescence on skeletal muscle generation was assessed after shortterm and longterm cardiotoxin induced muscle injury.
Results: Analysis of chromatin organization in quiescent/activated muscle stem cells and myotubes revealed that quiescent muscle stem cells possess abundant amount of facultative heterochromatin. Activation of muscle stem cells leads to massive reduction of facultative heterochromatin resulting in a relative increase of constitutive heterochromatin in myonuclei. Quiescent muscle stem cells specifically express the histone methyltransferases Suv420h1 and Ezh1 while activated muscle stem cells are characterized by the expression of Suv420h2 and Ezh2. Genetic inactivation of Suv420h1 reduces facultative heterochromatin in adult muscle stem and leads to activation and repositioning of the MyoD locus to the nuclear core, which results in persistent activation of muscle stem cells eventually causing stem cell depletion and impaired long term muscle regeneration. Genetic reduction of MyoD expression rescues facultative heterochromatin formation and loss of muscle stem quiescence thereby restoring muscle regeneration in Suv420h1 mutants.
Conclusion: The study demonstrates that the histone H4K20 dimethyltransferase Suv420h1 controls quiescence of MuSC formation by promoting formation of facultative heterochromatin. Our findings reveal an epigenetic axis consisting of Suv420h1 MyoD, which actively regulates the quiescent state of MuSC by formation of fHC thereby guarding the stem cell pool over a lifetime. The work provides strong evidence for the concept that satellite cell quiescent state is not a default cellular state but is instead a cellular state that must be actively maintained and reestablished.
Keywords: Adult Stem Cells, Tissue Regeneration, Skeletal Muscle Regeneration, Epigenetic Control, Stem Cell Quiescence

Is-8: Molecular Control of Cardiomyocyte Proliferation, Remodeling and Regeneration

Braun T

Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
Email: Thomas.Braun@mpi-bn.mpg.de

Background: Mammalian cardiomyocytes proliferate during embryogenic heart development but loose their proliferative potential shortly after birth concomitant with morphological maturation and a profound switch of the cellular metabolism. We wanted to explore the impact of hypoxic signaling on cardiac progenitor cells and neonatal cardiomyocytes. Furthermore, we were interested to understand the circuits controlling dedifferentiation of cardiomyocytes with the aim to promote cardiac regeneration, a virtue that is rapidly lost after birth.
Materials and Methods: We used a combination of *in vitro* and vivo methods, including generation of different gain and

loss of function transgenic mouse strains, for manipulation of different cellular signaling pathways. Effects of genetic manipulations or pharmacological agents were analyzed using various omics technologies, as well as morphological and physiological assessments

Results: We found that cells in the mouse heart tube are hypoxic while Isl1+ cardiac progenitor cells (CPCs) in the secondary heart field (SHF) are normoxic. Induction of hypoxic responses in Isl1+ CPCs causesd congenital heart disease by repression of Isl1 and activation of Nkx2.5, which results in decreased cell proliferation and enhanced cardiomyocyte specification. Interestingly, HIF1α forms a complex with HES1 and SIRT1 at the Isl1 gene, which represses Isl1 in the hypoxic heart tube or when ectopic hypoxic responses are induced. Subsequently, reduced Isl1 expression abrogates ISL1 dependent recruitment of HDAC1/5 inhibiting Nkx2.5 expression. Inactivation of Sirt1 in Isl1+ CPCs prevents CHDs induced by pathological hypoxia through inhibition of Isl1 suppression via the HIF1α/HES1/SIRT1 complex. Furthermore, we discovered that microRNAs are instrumental for suppression of two crucial regulatory circuits controlling postnatal cardiomyocyte proliferation and dedifferentiation, the FGFR and OSMR pathways. Concomittant inactivation of both miR gene clusters in postnatal cardiomyocytes resulted in activation of stem cell markers, expression of cell cycle regulatory genes and cellcycle reentry of adult cardiomyocytes. Importantly, inhibition of FGFR and OSMR signaling pathways reversed most effects of miR1/133a depletion on the cell cycle of cardiomyocytes while longterm dedifferentiation of cardiomyocytes compromised cardiac function resulting in heart failure.

Conclusion: Our results indicate that spatial differences in oxygenation of the developing heart serve as signals to control CPC expansion and cardiac morphogenesis. We propose a model that connects physiological hypoxia to homeostasis of CPCs and explains mechanisms underlying some nongenetic causes of CHD. In addition, we propose a crucial role of miRs in maintaining the postmitotic differentiated state of cardiomyocytes and suggest a negative feedback loop restricting dedifferentiation.

Is-9: Epigenetic Regulation of Skeletal Muscle Stem Cells

Braun T

Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
E-mail: Thomas.Braun@mpi-bn.mpg.de

Background: Skeletal muscle contains Pax7 expressing muscle stem or satellite cells (MuSC) enabling muscle regeneration throughout most of adult life but the molecular circuits controlling muscle stem cell maintenance, activation, and differentiation are only partially understood. During activation of MuSC, organization of the chromatin undergoes dramatic changes resulting in a massive loss of heterochromatin. We ought to understand whether changes in the global chromatin organization plays a major role for the regulation of MuSC.

Materials and Methods: We used a combination of *in vitro* and vivo methods, including generation of different gain and loss of function transgenic mouse strains and largescale screening formats, for manipulation of different cellular signaling pathways. Effects of genetic manipulations or pharmacological agents were analyzed using various omics technologies, as well as morphological and physiological assessments.

Results: We have conducted a large highresolution mass spectrometry based analysis of proteins expressed in satellite cells combined with a non biased high throughput lentiviral RNAi screen. Among numerous other important classes of proteins we detected several chromatinmodifying enzymes, which seem to play a pivotal role in the regulation of satellite cell activation and proliferation. We found that satellite cell specific inactivation of arginine methyltransferase Prmt5 completely prevents proliferation of muscle stem cells resulting in successive depletion of muscle stem cells during Prmt5. Further characterization of other chromatin modifying enzymes specifically expressed in satellite cells and myofibers unveiled that skeletal muscle stem cell primarily carry facultative heterochromatin, which after differentiation of satellite cells to myofibers switches to a combination of euchromatin and constitutive heterochromatin. Inactivation of the chromatin modifier Suv420h1 in muscle stem cells results in widespread chromatin rearrangements and loss of PCR dependent H3K27me3 modifications, which causes relocation of the MyoD locus from the nuclear periphery and precocious MyoD expression.

Conclusion: Our results indicate that global rearrangements in chromatin organization play a major role for the regulation of MuSC quiescence. Epigenetic modifiers are pivotal for the control of various steps of the stem cell cycle. We reason that Prmt5 generates a poised state, which keeps MuSC in a standby mode thus allowing rapid MuSC amplification under disease conditions. Our findings also revealed an epigenetic axis consisting of Suv420h1 and MyoD, which actively regulates the quiescent state of MuSC by formation of facultative heterochromaton thereby guarding the stem cell pool over a lifetime. In contrast, formation of constitutive heterochromatin, which mainly occurs at subtelomeric and pericentromeric regions, appears to assure genomic stability of muscle stem cells.

Is-10: Engineering The BioInterface at The Nanoscale

Didar T

Department of Mechanical Engineering, School of Biomedical Engineering, McMaster University, Ontario, Canada
Email: didart@mcmaster.ca

The biological/nonbiological interface system is an important cornerstone for the fabrication of a wide range of biomedical devices. Platforms as diverse as labonchip and pointofcare diagnostics, 3D tissue culture scaffolds, organsonchips and implants all rely on the effective interaction of cells and/or biorecognition elements (proteins/peptides, enzymes, antibodies, etc.) with nonbiological surfaces. I will present an overview of our research on micro/nanoscale design of novel biomedical coatings and their integration into medical devices such as catheters, vascular grafts and extracorporeal circuits as well as flexible sensing interfaces. More specifically I will discuss our recent results on design and development of omniphobic coatings with simultaneous repellency and targeted binding of desired biological species where biofouling and coagulation is minimized.

Is-11: Miniaturized Platforms for High Throughput Point of Care Diagnostics, Cell Sorting and Organs On Chips

Didar T

Department of Mechanical Engineering, School of Biomedical Engineering, McMaster University, Ontario, Canada
Email: didart@mcmaster.ca

Miniaturized platforms such as micro/nano patterned interfaces and microfluidic devices provide powerful tools to study biological phenomena at micro and nano scale and to develop novel technologies for a variety of biomedical applications such as point of care diagnostics, cell sorting and dug discovery. I will present an overview of our research on design, fabrication and embedding of biofunctional interfaces in microfluidic chips as well as large scale microfluidic systems and will discuss their applications for detection and sorting of rare cells, drug testing, organsonchips and therapeutics. I will present several microfluidic designs for labelfree and high throughput separation, *in vitro* patterning and culture of rare and primary cells followed by developing organsonchips platforms for *in vitro* disease modeling and drug discovery.

Is-12: Dissecting MSC Limitations to Design A More Efficient Regenerative Medicine for the Skeleton

Dominici M

Department of Medical and Surgical Sciences for Children and Adults, University Hospital of Modena and Reggio Emilia, Modena, Italy
Email: massimo.dominici@unimore.it

Background: Our group in collaboration with others developed MSC based strategies to challenge their skeletal regenerative potential. During these studies several goals were achieved and, at the same time, limitations emerged, in particular when pushing MSC properties towards clinics. Thus, we wanted to better understand these limitations with the aim to minimize them within more efficient regenerative medicine approaches in orthopaedics.
Materials and Methods: We isolated MSC from different donors and assessed their *ex vivo* performance parameters (i.e proliferation, differentiation, senescence) in combination with extensive mRNA and miRNA expression profiling.
Results: We first understood and confirmed the existence of variability in the bone regenerative potentials of MSC suggesting the need of more indepth investigations/explanations. Thus, we conceived the introduction of combinatory assays capable to predict bone formation within defined MSC populations. We additionally focused on agedependent difference in MSC performance, providing evidence of molecular signature related with both proliferation and differentiation. Transferring this concept into tissue regeneration, we took those molecular determinants of performance to increase MSC regenerative potentials obtaining unexpected results. This strategy originated novel approaches to predict and enhance pivotal MSC features, such as proliferation and skeletal regeneration, further providing insights on stem cells aging.
Conclusion: Recognizing and understanding limitations in MSC "*ex vivo* life" could generate more optimized therapeutic tools in regenerative medicine for skeletal disorders and more.

Is-13: Modifying MSC to Target Cancers

Dominici M

Department of Medical and Surgical Sciences for Children and Adults, University Hospital of Modena and Reggio Emilia, Modena, Italy
Email: massimo.dominici@unimore.it

Background: We and others uncovered that MSC can be redirected to target tumors becoming powerful delivering tools for anticancer molecules that are generally characterized by suboptimal bioavailability after systemic infusion. Focusing on death ligands and their potential in inducing selective cancer death, we want to deliver tumor necrosis factor related apoptosis inducing ligand (TRAIL) to different cancer models, in particular to both pancreatic adenocarcinoma and to sarcomas.
Materials and Methods: We armed adipose MSC with retro and lentiviral vectors to target a large variety of tumor lines and primary cancer cells both *in vitro* and *in vitro*. Results: We show that MSC can successfully deliver TRAIL variants to rapidly induce tumor death in several cancer settings. We additionally demonstrate that MSCdelivered TRAIL variants can induce caspase activation even in rhTRAIL highly resistant cell lines, indicating that the celldelivery is providing an added value to the cytotoxic potential of this well know deathligand.
Conclusion: We are now translating these technological platforms into a clinical scenario starting from patients with locally advanced inoperable pancreatic adenocarcinoma.

Is-14: *In Vitro* Generation of Meiosis-Competent Germ Cells from Embryonic Stem Cells by Engineering The Delivery of BMP4

Esfandiari F

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, Cell Science Research Center, ACECR, Tehran, Iran
Email: esfandiari65f@gmail.com

Background: Germ cells (GCs) are responsible for fertility in multicellular organisms. 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 no gametes.In this regard, new therapeutic approaches should be explored for infertility treatment, which necessitates an indepth understanding of GC development and function. In recent years, embryonic stem cells (ESCs) have shown their capability to differentiate along tissuespecific lineages. Of particular interest, derivation of GCs from ESCs provides an unlimited source with which to generate functional gametes for infertile couples as well as exploring principles that underlie reproduction. During embryonic development primordial germ cells (PGCs), the founders of GCs, are specified from the proximal epiblast by receiving bone morphogenetic protein 4 (BMP4) from extraembryonic ectoderm. Accordingly, mouse ESC–derived epiblast-like cells (EpiLCs) have been induced to primordial GC-like cells (PGCLCs) by addition of soluble BMP4.The resultant PGCLCs had the ca-

pability to restore spermatogenesis in infertile mice and contributed to healthy offspring. However, inability of PGCLCs to go through meiosis *in vitro* has remained a major challenge. In this study, a novel approach is presented for generation of GCs from ESCs. In the present study, we hypothesized that MP delivery of BMP4 inside the EpiLCs aggregates may lead to a more homogenous differentiation and produce PGCLCs that are more prone to go through meiosis *in vitro*.
Materials and Methods: To address our hypothesis, we have produced alginate sulfate MPs which provided affinity sites for loading and sustained release of BMP4. The BMP4 laden MPs were mixed with mESC derived EpiLCs to form MP incorporated aggregates.We analyzed gene and protein expression in PGCLCs which are produced by our engineering approach in order to investigate the efficiency of PGCLC formation. In order to investigate the potential of PGCLCs for go through meiosis, we generated and established a transgentic ESC line that express Stra8 (express when germ cells enter meiosis) upstream to red fleurescent protein (RFP). Then we treated the *in vitro* produced PGCLC with retinoic acid for 5 days both in PGCLCs that were produced in our system and in conventional system.
Results: The results here show that BMP4 release from alginate sulfate MPs is significantly retarded by the sulfated groups compared to neat alginate. Then, BMP4 laden MPs are incorporated within the aggregates during differentiation of GCs from ESCs. It is observed that BMP4-laden MPs increase GC differentiation from ESCs at least twofold compared to the conventional soluble delivery method. Interestingly, following meiosis induction, Dazl, an intrinsic factor that enables GCs to enter meiosis, and two essential meiosis genes (Stra8 and Smc1b) are upregulated significantly in MPinduced aggregates compared to aggregates, which are formed by the conventional method.
Conclusion: In summary, we used BMP4 delivering MPs as an innovative strategy in producing PGCLCs from ESCs. Together, our data show that controlled delivery of BMP4 during ESC differentiation into GC establish meiosis competent GCs which can serve as an attractive GC source for reproductive medicine.
Keywords: Meiosis, Tissue engineering, Infertility, Germ cells

Is-15: Cancer Gene Therapy – New Concepts and Novel Strategies

Farzaneh F

Department of Hematological Medicine, The Rayne Institute, King's College Hospital NHS Trust, Division of Cancer Studies, London, UK
Email: farzin.farzaneh@kcl.ac.uk

With better understanding of the molecular pathology of cancer, and with the development of better strategies for the delivery of therapeutic genes, gene therapy of cancer has held the promise of a significant contribution to the clinical treatment of cancer for over two decades. However, until very recently this promise has been substantially unfulfilled. Some of the more recent innovations are now resulting in the rapid development of a host of novel therapeutic strategies. These include the delivery of tumour suppressor genes, antioncogenic regulatory RNA sequences, delivery of genes encoding drug converting enzymes for the *in vivo* synthesis of cytotoxic agents from their lesstoxic precursors, and the development and clinical use of oncolytic viral vectors including those with selective replication and lytic activity within the tumour cells. Other applications of gene therapy, include the development of genetically engineered, tumour targeted, immune cells with cytolytic activity specifically against cancer cells (e.g. CART cells). Recent developments in this array of gene therapy based strategies will be reviewed. Specific examples of the most promising new strategies, including the recently licensed gene therapy drugs, will be described.

Is-16: Therapeutic Cancer Vaccines

Farzaneh F

Department of Hematological Medicine, The Rayne Institute, King's College Hospital NHS Trust, Division of Cancer Studies, London, UK
Email: farzin.farzaneh@kcl.ac.uk

A large number of different classes of tumour associated antigens provide targets for immune recognition of malignantly transformed cells by the immune system, and for immune therapy of cancer. These antigens include viral gene products expressed by oncogenic viruses such as human papilloma virus (HPV), there is also a whole array of cancer associated mutations, abnormal glycosylation products, as well as ectopic and/or elevated expression of such proteins in different malignancies. In addition to these common antigenic targets, recent data has demon strated the presence of other, entirely patient and tumour specific mutations. Both the common and the 'private' tumour associated antigens provide potential targets for the immune mediated therapy of cancer. Such immune therapy strategies are of particular relevance to the eradication of the residual cancer cells, most importantly the cancer stem cells, which can contribute to the relapse and recurrence of cancer, despite a successful initial response to therapy.
In this presentation some of the most prominent of the new cancer immune therapy strategies, will be reviewed. New strategies for vaccination mediated induction of antigen specific cellular immunity, against both the common oncogenic targets and the unique patient and tumour specific neoantigens will be described.

Is-17: Three Dimensional (3D) Organoid Cultures of The Pancreas as A Mean to Study Pancreas Development and Diseases

Figueiredo Larsen M[1], Steffenssen SB[1], Greggio C[2], De Franceschi F[2], Yennek S[1], Nakamura A[1], Gonçalves C[1], Larsen M[1], Lütolf M[2], Grapin Botton A[1, 2*]

1. DanStem, University of Copenhagen, Copenhagen, Denmark
2. ISREC, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
Email: anne.grapin-botton@sund.ku.dk

Background: Our goal was to develop culture methods that enabled to study mouse and human pancreas organogenesis and diseases *in vitro*.
Materials and Methods: To investigate how single cells exchange information and form a community, we have setup a method to grow dissociated pancreas progenitors in 3D. We culture the cells embedded in Matrigel or other polymers. We

have designed two culture media that either enable the maintenance of progenitors, or their differentiation and selforganization into a structure resembling the pancreas. The number of seeded cells, the chemicals added to the medium and mechanical parameters can be controlled and tested. The cultures can be monitored by live imaging.
Results: Using mouse pancreas progenitors, we have observed that small groups but not single cells expand, differentiate and selforganize in culture to form organoids of thousands of cells that reproduce many features of the pancreas. We find that heterogeneity in Notch/delta signaling in progenitors is at least in part responsible for the community effect and drives progenitor expansion. The talk will focus on selforganization, especially when and how heterogeneity between cells appears. We will also discuss how two different media enable different culture states to be installed. We will also report on recent advances in establishing a similar culture system starting from human embryonic stem cells.
Conclusion: Though focused on one organ, the pancreas and its embryonic development, the approaches we have used are relevant to other organs, including organ homeostasis in adults and analysis of clonal diversity in tumors. Such 3D culture systems now enable to grow cells that could not previously be grown *in vitro* and will enable to address questions *in vitro* using cell types more relevant than cell lines.

Is-18: Diet, Inflammation, and Stem Cells: Trading off Regenerative Response with Cancer Risk

Fodde R

Department of Pathology, Rotterdam, Zuid Holland, Netherlands
Email: r.fodde@erasmusmc.nl

Background: The recent debate on the relative importance of environmental vs. intrinsic factors in cancer onset raised many concerns because of fears that the general public might conclude that cancer prevention programs are not worthwhile the effort. It was proposed that most cancer cases can be explained by the high number of stem cell divisions in the tissues where they arise and by the consequentially increased chance of accumulating mutations in cancerrelated genes. Independent studies, however, reached radically different conclusions: exposure to environmental risk factors represents an essential requirement for cancer. Of note, the intrinsic rate of stem cell division and the environmental factors cannot be regarded as independent variables as it is plausible that extrinsic factors do affect stem cell homeostasis. Colon cancer, arising in one of the most proliferative and selfrenewing tissue in our body was indisputably shown to have a strong environmental component with western style dietary habits and inflammation among the major risk factors. According to our main hypothesis, dietary and inflammatory colon cancer risk factors act primarily on the stem cell niche by introducing alterations of both of quantitative and qualitative nature as they not only expand subpopulations of stem and progenitor cell targets for tumor initiation, but also induce dedifferentiation and activate novel stem cell types in response to stress signals.
Materials and Methods: To test our main hypotheses, we take advantage of two distinct mouse models of colon cancer due to the exposure to 1. a "westernstyle" diet (NWD1); and 2. to DSSsupplemented drinking water. The latter result in the chronic inflammation in of the murine GI tract, reminiscent of inflammatory bowel disease in man. Monitoring and lineage tracing analysis of stem and niche cells is performed throughout the intestinal tract on different CreLox models for stem and niche cells, fed with the experimental diets, and with the inflammation inducer DSS. Global and more functional analyses will be employed.
Results: 1. Lgr5+ stem cell function is abrogated by a sporadic colon cancer inducing western style diet. Both the proliferative capacity and stem function of Lgr5+ cells were abrogated in NWD1fed animals. 2. The overall intestinal stem cell function is enhanced in mice fed with a Westernstyle diet, an effect mediated by Paneth cells. 3. Secreted phospholipases are key intestinal stem cell factors in homeostasis, inflammation and cancer. 4. A metabolic dichotomy earmarks the intestinal stem cell niche. Whereas Lgr5+ cells display high mitochondrial activity, Paneth cells are earmarked by glycolysis as the main metabolic activity. Inhibition of mitochondrial activity or of glycolysis.
Conclusion: Our adult tissues react to specific factors as dietary nutrients or inflammation through a regenerative response that involves different cellular identities including stem and fully differentiated niche cells. Activation of these distinct cellular lineages is beneficial for the repair of the damaged tissue though at the cost of an increase of cancer risk due to the expansion of specific subpopulations of cell targets for tumor initiation and progression, but also to dedifferentiation and reprogramming of other.
Keywords: Colon Cancer, Western Style Diet, Inflammatory Bowel Disease, Stem Cell, Niche Cell

Is-19: Cytokines TNF-α, IL-6, IL-17F, and IL-4 Differentially Affect Osteogenic Differentiation of Human Adipose Stem Cells

Forouzanfar T[4], Bastidas Coral AP[1], Bakker AD[1], Zandieh Doulabi B[1], Kleverlaan CJ[2], Bravenboer N[3], Klein Nulend J[1*]

1. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije University, Amsterdam, Netherlands
2. Dental Materials Science, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije University, Amsterdam, Netherlands
3. Department of Clinical Chemistry, VU University Medical Center, Amsterdam, Netherlands
4. Department of Oral and Maxillofacial Surgery, VU University Medical Center, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands
Email: j.kleinnulend@acta.nl

Background: During the initial stages of bone repair, proinflammatory cytokines are released within the injury site, quickly followed by a shift to antiinflammatory cytokines. The effect of pro and antiinflammatory cytokines on osteogenic differentiation of mesenchymal stem cells is controversial. Here, we investigated the effect of the proinflammatory cytokines TNF-α, IL-6, IL-8, and IL-17F, and the anti-inflammatory cytokine IL-4 on proliferation and osteogenic differentiation of human adipose stem cells (hASCs).
Materials and Methods: hASCs were treated with TNF-α, IL-6, IL-8, IL-17F, or IL-4 (10 ng/ml) for 72 h mimicking bone repair.

Results: TNF-α reduced collagen type I gene expression, but increased hASC proliferation and ALP activity. IL-6 also strongly enhanced ALP activity (18-fold), as well as bone nodule formation by hASCs. IL-8 did not affect proliferation or osteogenic gene expression, but reduced bone nodule formation. IL-17F decreased hASC proliferation, but enhanced ALP activity. IL-4 enhanced osteocalcin gene expression and ALP activity, but reduced RUNX2 gene expression and bone nodule formation.
Conclusion: In conclusion, all cytokines studied have both enhancing and reducing effects on osteogenic differentiation of hASCs, even when applied for 72 h only. Some cytokines, specifically IL-6, may be suitable to induce osteogenic differentiation of mesenchymal stem cells as a strategy for enhancing bone repair.
Keyword: Adipose Stem Cells, Mesenchymal Stem Cells, Osteogenic Differentiation, Cytokines, Bone Repair

Is-20: RNA-Directed Programming of Embryonic Stem Cell

Ghanbarian H

Department of Biotechnology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: hghanbarian@sbmu.ac.ir

Background: We reported earlier that microinjection of small non-coding RNAs is associated with epigenetic modifications and results in transcriptional activation of specific target genes. Whether epigenetic mechanisms are involved in the initial determination of gene expression in the early embryo is an important question. Differentiation of cardiomyocytes is an early event during embryogenesis *in vivo*, which can be monitored by the appearance of beating cells in cultures *in vitro*. To promote cardiac differentiation of ES cells, we attempted to modulate expression of Cdk9, one of the main actors of cardiac differentiation *in vivo*. To determine the molecular mechanisms involved and to explore whether such epigenetic regulations could play a role in early development, we used a cell culture system as close as possible to the embryonic state. We report efficient cardiac differentiation of embryonic stem (ES) cells induced by small non-coding RNAs with sequences of Cdk9, a key player in cardiomyocyte differentiation.
Materials and Methods: Mouse AB1 ES cells were grown on mouse embryonic fibroblast (MEFs) feeders in standard ES culture medium. RNA was extracted using the Trizol Reagent (Invitrogen). 0.5 µg RNA samples were reverse transcribed to cDNA using random hexamer primers and MLV reverse transcriptase (Invitrogen). q-PCR was performed using the 'Platinum® SYBR® Green qPCR SuperMix-UDG' kit (Invitrogen). Runon Assay was performed as described in the manufacturer instructions. Total lysates from cell cultures were prepared, electrophoresed, and blotted as described. Northern blot analysis was performed according to standard methods. Chromatin immunoprecipitation (CHIP) assay was carried out according to the protocol of the ChIP Assay Kit (Millipore cat. 17-295). According to standard methods, ES cells were injected in 3.5 days blastocysts after electroporation of Cdk9 sense transcript fragment (Cdk9-f: 5'-GAUUUUCUCCUCCAGUACAUAU-3'), or microRNA-1 (miR-1: 5'-UGGAAUGUAAAGAAGUAUGUAU-3'), or a pIRESneo-EGFP DNA/miR-1 construct. As controls, blastocysts were microinjected with mock-electroporated ESCs.
Results: To investigate whether Cdk9 target mRNAs induce transcriptional variation in cell types, mouse embryonic stem cells were analysed after electroporation of a 22nt oligoribonucleotide with a nucleotide sequence identical to that of the Cdk9 mRNA. Extracts prepared 48 hours after electroporation showed an increase in Cdk9 expression. Strand specific RT-PCR assays confirmed the presence of transcripts complementary to the most 3' region of the mRNA. oligonucleotides with either an intronic sequence of Cdk9 or the exonic sequence in the 3' region induced the transcriptional activation of Cdk9. Both correspond to regions in which antisense transcripts are detected. Conversily, when electroporated into Agodeficient ES cells, the transcript fragment did not induce an increase in Cdk9 expression. Interestingly, Cdk9 felectroporated ES cells differentiated faster and more efficiently into cardiac muscle cells than the original ES line. Moreover, injection of miR-1 or Cdk9-felectroporated ES cells into blastocysts resulted in increased expression of Cdk9 in embryonic hearts at E18.5.
Conclusion: Cdk9 transcript derived oligoribonucleotides are capable to induce Cdk9 expression in different cell systems. Requirements for Argonaute proteins and for endogenous antisense transcripts at the locus indicate that the inducer oligoribonucleotides are processed by the RNAi machinery. Induction of Cdk9 resulted in efficient cardiac differentiation of ES cells *in vitro*. Injection of Cdk9-felectroporated ES cells into blastocysts induced cardiac growth indicating that RNA-programmed ES cells contribute specifically to the heart *in vivo*.
Keywords: Antisense Non-Coding RNA, Embryonic Stem Cells, Locus Specific Induction, Cdk9, Cardiac Differentiation

Is-21: Development of Cellular Therapies – Lessons Learned from T Lymphocytes

Gee AP*, Leen A

Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas, USA
Email: apgee@txch.org

Cellular therapies, including those employing stem cells, are showing great promise for treatment of a wide variety of diseases. T lymphocytes, genetically engineered to express a chimeric antigen receptor (CAR), have been particularly effective in patients with leukemia. This success partly has its origins in the development of T cell therapies for viral infections, and for lymphomas of viral etiology. The evolution of this work over more than 25 years, has provided a number of valuable insights into the scientific and technical challenges inherent in bringing any cellular therapy into clinical trials. These occurred at all stages of development and included generating sufficient numbers of cells, broadening their viral specificity, developing culture vessels, adapting the concept to improve CAR T cell efficacy, and dealing with regulatory authorities to obtain the required approvals. In this presentation, these challenges, and our approaches to overcoming them, will be reviewed. The hope is that our experiences, both good and bad, will provide useful lessons to others working in this exciting new field of medicine.

Is-22: Transitioning Cellular Therapies from Research into Clinical Trials: The Baylor Experience

Gee AP

Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas, USA
Email: apgee@txch.org

The Center for Cell and Gene Therapy at Baylor College of Medicine is currently conducting more than 40 clinical trials using cell therapy products, including virus specific T cells, CAR T-cells, and NK cells. In addition, it provides mesenchymal stromal cells for regenerative studies in cardiology and neurology, and for immune conditioning of kidney transplant patients. To prepare for each of these trials we must transition research based methods into those that are compliant with U.S. Current Good Manufacturing Practices. This involves two major activities, the first of which is to develop a manufacturing procedure that will provide sufficient cells of the required quality under conditions that meet U.S. regulations. The second is to work with the Food and Drug Administration to ensure that this manufacturing procedure will be acceptable for use in the proposed clinical trial, and that the appropriate tests will be used to release the cells for clinical use. Following FDA approval, the next major activity is to manufacture the cellular product for patients entered into the various trials. Each of these activities has its particular challenges, especially for newer centers, and by sharing these, we hope to speed the entry of new products into clinical studies.

Is-23: Treating Patients with Naïve T Cell-Derived Antiviral T Cells

Hanley PJ

Laboratory Facility Director Cellular, Therapy and Stem Cell Processing, The George Washington University, USA
Email: phanley@childrensnational.org

Hematopoietic stem cell transplantation (HSCT) is the only curative option for genetic disorders and high risk or relapsed malignancies. The necessary conditioning regimens, however, leave recipients susceptible to infections – most notably viral infections from viruses like Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus (AdV). Although antiviral pharmacotherapies exist for CMV and EBV, they are not always effective, can have serious side effects in patients trying to engraft a new immune system, and no proven therapy is available for Adenovirus. The *ex vivo* expansion of T cells targeting viruses, however, has shown to be a potential therapy for viral infections in transplant recipients, even in those who have already failed pharmacotherapy. In fact, as a testament to the need for new therapies, roughly half of the patients we have treated with our T cell therapies have come from outside institutions such as the University of Pennsylvania and Johns Hopkins University after failing conventional therapies and lacking additional options.
Moreover, we have infused over 30 patients with our T cell therapies and in the setting of our antiviral T cells from CMVseropositive donors, we have seen an overall response rate over>80%. The limitation of this approach, however, is that the stem cell transplant recipients at the greatest risk for CMV infection are recipients receiving a stem cell transplant from a CMVseronegative donor, because there are no CMV-specific T cells present in the stem cell graft to confer protection against the virus. Therefore, we developed a system to generate CMV-specific T cells from two sources of CMV-naïve T cells: umbilical cord blood and CMV-seronegative adult donors. Interestingly, naïve T cell-derived CMV-specific T cells recognize atypical epitopes of CMV when compared to memory derived T cells. Nevertheless, using deep T cell receptor sequencing and ELISPOT assay, we are able to detect infused virus specific T cells in the peripheral blood of patients as late as 1 year post-T cell infusion in many of the 13 patients that have been infused to date.
In conclusion, we have established a new cell therapy program at Children's National where we offer a range of antiviral and antitumor T cells for patients in need of novel therapies and in collaboration with the Center for Cancer and Immunology Research and Division of Blood and Marrow Transplantation, continue to develop innovate therapies at the bench that we are able to translate to the bedside.

Is-24: Requirements for A Cellular Therapy Facility: Expecting The Unexpected

Hanley PJ

Laboratory Facility Director Cellular, Therapy and Stem Cell Processing, The George Washington University, USA
Email: phanley@childrensnational.org

Cell therapy has shown promise for countless diseases and has made tremendous progress in the past 20 years. Virus specific T cells, mesenchymal stromal cells, NK cells, chimeric antigen receptor bearing T cells and other therapies have been developed as alternatives to pharmacotherapy, chemotherapy, and radiation. As a result, there has been an increase in demand for specialized cell therapy facilities worldwide. However, what a cell therapy facility is and what it entails is difficult to appreciate until one is forced to develop it. Here we will discuss what to expect, such as the facility itself, staff, costs, quality management, and time. Beyond the expected, we will identify the unexpected elements of a cell therapy facility, including space to store excessive documentation, the need for regulatory staff, the translation of research products into the clinic, time needed in the facility, and how to ensure that your facility is being cleaned by staff not under your control.

Is-25: Axon and Dendrite Formation, The Molecular Mechanism Viewed from Membrane Trafficking

Hisanaga SI

Laboratory of Molecular Neuroscience, Department of Biological Sciences, School of Science and Engineering, Tokyo Metropolitan University, Hachioji, Japan
Email: hisanaga-shinichi@tmu.ac.jp

Neurons extend two different processes called axon and dendrites, through which neurons communicate each other. In general. axon is a single long process sending the excitation signal to other neurons, and dendrites are multiple and branching processes, where neurons receive synaptic stimuli from other neurons. It is quite important to establish two types of neurites to the correct target at the exact time and speed. Since axon and

dendrite formation can be captured in the culture of primary neurons, extensive studies have been performed. It is known well the role of cytoskeletons in neurite formation. In contrast, little is understood how membrane trafficking contributes to neurite outgrowth, although the membrane supply appears to be a critical step for neurite outgrowth because the surface expansion should accompany with the elongation of neurites. We recently found that recycling endosomes are a major membrane vesicle participating in the supply of the membrane components. The transport of recycling endosomes is regulated by Rab11 GTPase. We identified two protein factors, LMTK1A and GRAB, which control the Rab11 activity at its upstream and downstream respectively. LMTK1 regulates Rab11 negatively as evidenced from its knockdown leading to enhanced neurite outgrowth, and GRAB mediates the membrane trafficking signal from the Rab11-dependent recycling endosomes to the Rab8-dependent exocytic process. I would like to discuss how LMTK1 or GRAB regulates the neurite outgrowth through the Rab activity in neurons.

Is-26: Cyclin Dependent Kinase 5, Its Role in Neuronal Differentiation, Synaptic Activity and Neurodegeneration

Hisanaga SI

Laboratory of Molecular Neuroscience, Department of Biological Sciences, School of Science and Engineering, Tokyo Metropolitan University, Hachioji, Japan
Email: hisanaga-shinichi@tmu.ac.jp

Cyclin dependent kinase 5 (Cdk5) is a member of Cdk family, most of which are activated by binding cyclin activation subunits and promote cell cycle progression in proliferating cells. In contract, Cdk5 is a unique member of Cdks, displaying the activity in postmitotic neurons. This is because Cdk5 activators, p35 and p39, are predominantly expressed in neurons. While cycling Cdks promote cell cycle in nucleus, the active Cdk5 is anchored to cytoplasmic membranes. This membrane binding is mediated by myristoylation of p35. Cdk5 is a multifunctional kinase, playing a variety of roles in whole long life of neurons from differentiation to cell death. In particular, it is known that Cdk5-deficient mice show inversion of neuronal cell layers in several brain areas including cerebral cortex and hippocampus, indicating its role in migration of newborn neurons. Cdk5 also regulates neurite outgrowth via neuronal cytoskeletons and membrane trafficking. Cdk5 determines the threshold of synaptic transmission at both the presynaptic and postsynaptic regions by suppressing excitation. We think Cdk5 is a house keeping protein kinase, different from other many protein kinases, which are activated by external stimuli. Proper regulation of Cdk5 activity is required for normal neuronal activity but its degradation by cleavage of p35 to p25 with calpain induces neurons death, causing neurodegenerative diseases. I will introduce the mechanism regulating the Cdk5 activity and several Cdk5 functions in neurons.

Is-27: Liposomes Encapsulating Alendronic Acid for Γδ T Cell Cancer Immunotherapy

Hodgins N[1], Wang J[1], Parente Pereira A[2], Al-Jamal WT[3], Maher J[2], Al-Jamal KT[1*]

1. Institute of Pharmaceutical Science, King's College London, UK
2. CAR Mechanics Group, Research Oncology, Guy's Hospital, London, UK
3. School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, UK
Email: khuloud.al-jamal@kcl.ac.uk

Background: Nitrogen-containing bisphosphonates (N-BPs) including alendronic acid (ALD) inhibit farnesyl diphosphate synthase (1-3), and sensitise tumour cells to destruction by Vγ9/Vδ2 T cells. N-BPs have limited *in vivo* activity due to rapid clearance from the circulation. Liposomes lead to increased levels of N-BPs at tumour sites and can be used with Vγ9/Vδ2 T cells for cancer immunotherapy. We hypothesise that uptake of ALD liposomes (L-ALD) and Vγ9/Vδ2 T cells differs among tumour models. This work aims at quantifying the amount of L-ALD and Vγ9/Vδ2 T cells in different tumour models in order to assess their suitability for this immunotherapy.
Matherials and Methods: L-ALD were formulated with DSPE-DTPA (1, 2-distearoylsnglycero-3-phosphoethanolamine N-diethylenetriaminepentaacetic acid), and radiolabelled with Indium-111 (111In). Vγ9/Vδ2 T cells were isolated from whole blood and radiolabelled with 111In tropolone. Immunocompromised mice were inoculated with the melanoma cell line A375Pβ6 to form subcutaneous, intraperitoneal or lung tumours. The biodistribution of intravenously injected L-ALD or Vγ9/Vδ2 T cells in these different tumour models implanted in NSG mice, was assessed by gamma counting. Tumour therapy study was performed in the pseudo metastatic A375Pβ6 lung mice models. Mice were injected i.v. with L-ALD (0.5 μmol ALD/mouse) followed by Vγ9/Vδ2 T cells (1×10^7 cells/mouse) with a 24 h interval between both treatments. This treatment cycle was repeated 3 times, at weekly intervals. Uninjected mice or mice injected with L-ALD or Vγ9/Vδ2 T cells were used as controls.
Results: *In vivo*, Inlabelled liposomes have shown accumulation of 1.9, 5.2 and 1.9 % injection dose per gram (% ID/g) in subcutaneous tumours, intraperitoneal tumours and in tumour bearing lungs, respectively. In the same three tumour models, 111Inlabelled Vγ9/Vδ2 T cells had accumulation of 1.0, 1.4 and 22.5 % ID/g, respectively. Preinjection of mice with free ALD or L-ALD, did not lead to a significant change in the tumour accumulation of Vγ9/Vδ2 T cells. *In vivo* tumour therapy study showed significantly delayed tumour growth delay in mice injected with L-ALD and by Vγ9/Vδ2 T cells but not in any of the other treatment groups.
Conclusions: The location of the tumour influences the %ID/g of both L-ALD and Vγ9/Vδ2 T cells. The amounts reaching lung tumours are still able to result in improved therapeutic efficacy, when immunotherapy is combined with chemotherapy. Future work will focus on performing therapy studies in intraperitoneal tumour mice model, and on further improving the treatment efficacy by active targeting of L-ALD to receptors overexpressed in cancer cells. The results confirmed the applicability of this chemotherapy/immunotherapy combinatory approach to treat metastatic cancer. It also shed light on the importance of quantifying the nanocarrier and the cellbased therapeutic agent, in tumours of different locations, when designing clinical studies for human patients.

Is-28: Engineering Carbon Nanoneedles for Double Stimulation of Dendritic Cells *In Vivo*

Hassan HAFM1, Smyth L[1], Summers HD[2], Lombardi G[1], Al-Jamal Kh[1*]

1. Institute of Pharmaceutical Science, King's College London, London, UK
2. College of Engineering, Swansea University, Swansea, UK
Email: khuloud.al-jamal@kcl.ac.uk

Dendritic cells (DCs) are antigen presenting cells that recognise and present the introduced antigens to the immune cells to elicit an immune response. Multiwalled carbon nanotubes (MWNTs) are novel nanoscopic delivery systems that have superior cell internalisation properties compared to other delivery systems. Nanotubes have the ability to access the intracellular compartments via endocytosis or through direct translocation across the cell membrane, referred to as the nanoneedle mechanism. Reported studies have demonstrated the ability of MWNTs to improve the antigen delivery to DCs compared to the soluble antigen forms. However, none of the studies investigated system atically the effect of their length and surface charge on the interaction with DCs, and the immune response elicited. In this study, MWNTs were chemically functionalised using various approaches then conjugated to ovalbumin (OVA), used as a model antigen. The impact of varying MWNTs-OVA length and surface charge on the extent of DCs uptake, and the *in vitro* immune response intensities, using CD4+ and CD8+ T cells, were studied. These findings provide a better understating when selecting the optimal MWNTs physicochemical properties, for the induction of the most potent immune response by DCs.

Is-29: Muscular Dystrophies: How Could Stem Cells Help?

Karaoz E

Department of Histology and Embryology, Center for Regenerative Medicine and Stem Cell Research and Manufacturing (LivMedCell), Faculty of Medicine, Istinye University, Istanbul, Turkey
Email: ekaraoz@hotmail.com

Is-30: Cellular and Acellular Therapies in Neurodegenerative Diseases

Karaoz E

Department of Histology and Embryology, Center for Regenerative Medicine and Stem Cell Research and Manufacturing (LivMedCell), Faculty of Medicine, Istinye University, Istanbul, Turkey
Email: ekaraoz@hotmail.com

Is-31: Studying Single Cell Contribution to Organogenesis, The Pancreas Example

Kim YH[1], Larsen HL[1], Coll LM1, Rué P[2], Grapin Botton A[1*]

1. DanStem, University of Copenhagen, Copenhagen, Denmark
2. Department of Genetics, Wellcome Trust Centre for Stem Cell Research, University of Cambridge, Cambridge, UK
Email: anne.grapin-botton@sund.ku.dk

Background: Pancreas organogenesis relies on the expansion of progenitors and their differentiation into acinar, ductal and endocrine cells. Our study addresses whether the contribution of individual progenitors to organogenesis is heterogeneous in amounts of cells produced and cell types generated in the progeny.
Materials and Methods: We have investigated the contribution of individual progenitors to organogenesis at different times of development by tracking cells by 3dimensional live imaging, using *in vivo* clonal analysis and single cell PCR.
Results: Analyses after one division establish the role of symmetric renewing, symmetric differentiative and asymmetric divisions, and reveal stochasticity in endocrine fate commitment (Kim et al., PLOS Biology 2015). Analyses over several days address cumulative contributions and biases over multiple generations. These experiments show a great deal of heterogeneity in the size of clones and in the cell types single progenitors generate. In particular a great number of endocrine committed cells are identified, as well as bipotent endoductal) and tripotent progenitors (endoacinoductal) as soon as E9.5. We will discuss whether this is caused by molecular heterogeneity in the progenitor population, as analyzed by tracking specific populations and comparing with single cell expression data, or whether it reflects stochastic fate commitment. We will finally report on new data following differentiation paths in human stem cell derived pancreas progenitors.
Conclusion: These studies reveal that the development of the pancreas is driven by heterogeneous progenitors that form states rather than subtypes and that respond to regulative cues. They show that the niche occupied by the progenitor in the organ matters and pave the way to the identification of feedback signals ensuring that the pancreas is similar at the endpoint in different individuals.

Is-32: Patient Specific Hips Derived Cardiac Myocytes as Models for Inherited Cardiac Arrhythmia (LQT and CPVT)

Lipp P

Center for Molecular Signaling (PZMS), Center for Molecular Imaging and Screening, Institute for Molecular Cell Biology Research, Saarland University, Saarland, Germany
Email: Peter.Lipp@uks.eu

Background: Investigation of human induced pluripotent stem cell – derived cardiac myocytes (hiPS-CMs) as single cell models of human cardiac diseases.
Materials and Methods: We generated induced pluripotent stem cells (hiPSCs) from a CPVT1 patient carrying a novel ryanodine receptor type II (RyRII) S406L mutation and evaluated disarrayed intracellular calcium handling and action potentials (AP) by highspeed 2 dimensional confocal microscopy and patch clamp, respectively. From a LQT1 patient carrying a mutation in the KCNQ1 protein (R190Q) hiPS-CMs were generated. For these we virally expressed a genetically encoded voltage sensor to evaluate AP properties such as their duration. Additionally, an isogenic control line from the same patient was generated following correction of the LQT1 mutation by a homologous recombination approach. These myocytes were evaluated similarly to the former lineage.
Results: In CPVT1 hiPS-CMs, catecholaminergic stress resulted in disarrayed intracellular Ca handling and aberrant action potentials possibly due to an increased frequency and duration of elementary Ca release events (Ca sparks). Dantrolene restored normal Ca spark properties and rescued the arrhythmogenic phenotype. In LQT1 hiPS-CMs AP prolongation and fre-

quent early afterdepolarizations were evident in ventricular and atrial like, but not in nodal like myocytes when compared with their isogenic controls. Nevertheless, in these isogenetic myocytes a residual alteration of action potential properties was still remaining when compared to control cells.
Conclusion: Our data on the CPVT1 myocytes suggests defective interdomain interactions within the RYR2 channel as the underlying pathomechanism of this novel RyRII S406L mutation. The study on the LQT1 myocytes showed subtype specific expression of the diseasecausing gene that could be corrected genetically. In conclusion, hiPS-CMs can be employed as *in vitro* models for human cardiac diseases but additional patient-specific contributions have to be considered.

Is-33: Life Identification of Cellular Sub Types and Optical Action Potential Recording in Hips Derived Cardiac Myocytes

Lipp P

Center for Molecular Signaling (PZMS), Center for Molecular Imaging and Screening, Institute for Molecular Cell Biology Research, Saarland University, Saarland, Germany
Email: Peter.Lipp@uks.eu

Background: Develop an approach for lifeidentification of human induced pluripotent stem cell – derived cardiac myocytes (hiPS-CMs) subtypes with respect to action potential (AP) morphology.
Materials and Methods: We used cardiac lineagespecific promoters to drive the expression of a voltagesensitive fluorescent protein (VSFP-CR) in hiPSC-CMs, enabling subtypespecific optical AP recordings sequentially up to several days on the same cells. AP characteristics were analysed using ultrahigh speed imaging (500 frames/second) approaches.
Results: We employed Lentiviral gene transfer into hiPS-CMs and demonstrated subtype specific expression of a genetically encoded voltage sensor in nodallike (SHOX2 promotor), atriallike (sarcolipin promotor) and ventricular like (MLC2v promotor) myocytes. Subtypes were verified by either subtype specific markers or action potential shape. In addition, we confirmed the identity of individual cells by sequential single cell recording of action potentials (both, optically and electrophysiologically) followed by single cell PCR of marker gene transcripts.
Conclusion: For the first time, we demonstrate that a combination of FRET-based VSFP and CM subtype specific promoters allows selective optical AP measurements in ventricular, atrial, or nodal like hiPSC-derived CMs.

Is-34: Re-Myelination and Functional Integration of Ips-Derived Neural Precursors Following Transplantation in The Developing and Adult Demyelinated White Matter

Mozafari S

Paris School of Neurosciences (ENP), Pierre and Marie Curie (Paris VI) Brain and Spinal Cord Institute (ICM), Sorbonne University, France
Email:sabahmozafari@gmail.com

Induced pluripotent stem cell derived neural precursor cells (iPS-NPCs) may represent the ideal autologous cell source for cell based therapy to promote remyelination and neuroprotection in myelin diseases and can serve as suitable tools to model myelin disorders or to test the potential of pharmacological compounds. So far the therapeutic potential of these cells was approached in neonatal conditions. However, the repair efficacy and safety of these cells in the demyelinated adult central nervous system (CNS), a condition associated with decreased cell plasticity and scaring, remains to be well addressed. Moreover, whether the therapeutic behavior of these pluripotent derived cells resembles that of physiologically committed CNS-derived precursors remains elusive.
First, we used mouse iPS-NPCs and compared them sidebyside to embryonic CNS-derived cells, *in vitro* and *in vivo* after engraftment in models of adult spinal cord demyelination.
Using a very efficient and rapid technique our collaborators succeeded in bypassing the poor and slow differentiation process of human pluripotent cells into oligodendrocytes, *in vitro*. To validate the functionality and re/myelination potential of human iPS-derived glial precursors, we then transplanted them in newborn as well as adult models of dys/demyelination.
Our data from the first part, revealed the prominent capacity of survival, safe integration, migration and timely differentiation of the grafted cells into mature oligodendrocytes. Grafted cells generated compact myelin around host axons, restoring nodes of Ranvier and conduction velocity as efficiently as CNS-derived precursors while outcompeting endogenous cells. Moreover, we showed widespread migration, integration and extensive generation of functional human oligodendrocytes ensheathing host axons, forming compact myelin while reconstructing nodes of Ranvier both in newborn grafted and adult demyelination contexts. Our preliminary data showed that both mouse and human iPS-derived oligodendrocytes expressed Connexin 47 on their somata and in paranodes where they tightly connected to astrocytic Connexin 43. Together, these results provide novel and promising insights into the biology of skinderived reprogrammed cells (both mouse and human cells) in myelinaffected conditions and should help establishing the pertinence of using them for i. regenerative biomedicine of myelin diseases affecting the CNS or ii. modeling myelin disorders in vitro or *in vivo* in order to achieve personalized preclinical therapies for complex disorders of CNS myelin such as multiple sclerosis.

Is-35: A Solution for Cell Therapy Safety

Nagy A

Lunenfeld Tanenbaum Research Institute, Sinai Health System, Toronto, Canada
Email: nagy@lunenfeld.ca

Numerous human pluripotent stem cell based therapies are currently on their way to treat devastating degenerative diseases. However, concerns about the safety hold back the full utilisation of these promising new treatments. Here we introduce a concept and show the associated genome engineering strategy that addresses this issue and provides a solution for failsafe cell therapies.
To ensure the reliable expression of a suicide transgene system in proliferating cells, we transcriptionally linked it to a cell division essential endogenous locus (CDEL) in a homozygous manner. Our prototype suicide gene was the herpes simplex vi-

rusthymidine kinase (HSV-TK), and the prototype CDEL was Cdk1. The coding regions of these two kinases were connected with a viral 2A sequence by CRISPR/Cas9 assisted genome editing. We generated mouse and human embryonic stem cell lines with the above homozygous modification of the Cdk1 locus. Our results showed that we could ablate proliferating cells both *in vitro* and *in vivo* by ganciclovir treatment, the prodrug for HSV-TK. The elimination of proliferating cells could efficiently stop the growth of teratomas generated by these ES cells and rendered this tissue to dormancy.
Using published and our experimental measures of forward mutation rates, we defined the cell population based failsafe level for different genome alteration designs (genotypes). Then we mathematically modelled the probability of escape from our failsafe cell systems during expansion of cells to a number that might be needed for clinical use. Depending on the scenarios of different types of cell therapies, this concept in combination with another optional safety strategy developed in my lab will provide versatile options that meet the complex needs for the safety of future cell based therapies.

Is-36: Pluripotency in The Artificial Cell Space

Nagy A

Lunenfeld Tanenbaum Research Institute, Sinai Health System, Toronto, Canada
Email: nagy@lunenfeld.ca

The ability to reprogram somatic cells to a pluripotent state is paradigm shifting for both biology and medical research. Reprogramming continues to challenge many of our assumptions about the specification of cellular phenotypes, and yet, despite major efforts, we still lack a complete molecular characterization of the process. To address this gap, we generated a comprehensive molecular description of the reprogramming cascade toward two distinct pluripotent states. We explored alternative outcomes of somatic reprogramming by fully characterising reprogrammed cells independent of preconceived definitions of reprogrammed iPSC states. We demonstrate that manipulating the expression level of the reprogramming factor influences cells arrival to a non-ES cell-like or ES cell-like pluripotent state. This bifurcated process has been characterised with multiple "omic" platforms, consisting of the transcriptome (microRNA, lncRNA and mRNA), CpG methylation, ChIPsequencing (for chromatin marks: H3K4me3, H3K27me3 and H3K36me3), in addition to quantitative mass spectrometry profiling of the global and cell surface proteome. This dataset enables crossreferencing between "omic" platforms, which facilitates a deeper understanding of the cascade of molecular events driving the generation of pluripotent cells.

Is-37: Application of Mesenchymal Stem Cells in Liver Diseases: Current Landscape and Future Trends

Najimi M

Laboratory of Pediatric Hepatology and Cell Therapy, Institute of Experimental and Clinical Research (IREC), Université catholique de Louvain, Brussels, Belgium
Email: mustapha.najimi@uclouvain.be

Regenerative medicine is currently developed to provide repairing, substituting and regenerating solutions for tissues and organs altered both by disease and aging. This therapeutic approach is holding a great interest because its applicability may solve many current clinical problems such as organs shortage and transplant rejection. Among other emerging regenerative approaches, cell therapy development is offering innovative solutions for patients with unmet medical needs. Stem cells are developed as a major biotechnological alternative thanks to several competitive and distinctive advantages. Many liver defects are still untreatable while access to the current standard of care, orthotopic liver transplantation, is increasingly limited. Liver-cell based therapies are in progress to target acute, chronic and metabolic diseases. Adult mesenchymal stem/progenitor cells (MSC) are positioned as ideal candidates since they display unique ability of proliferation and differentiation into hepatocytic cells, resistance to cryopreservation, as well as immuno modulatory and safety profiles.
The presentation will appraise the accumulated knowledge with respect to preclinical and clinical use of MSC in liver disease settings. It will also discuss the challenging strategies currently investigated and willing to improve a standardized clinical development and widespread use of liver cell based therapies.

Is-38: Liver Mesenchymal Stem Cells for Liver Fibrosis

Najimi M

Laboratory of Pediatric Hepatology and Cell Therapy, Institute of Experimental and Clinical Research (IREC), Université catholique de Louvain, Brussels, Belgium
Email : mustapha.najimi@uclouvain.be

Chronic liver diseases represent the 7th cause of mortality worldwide and may have different etiologies including viral, autoimmune, drug induced, cholestatic and metabolic diseases. Liver fibrosis, which can evolve towards cirrhosis, is characterized by an accumulation of extracellular matrix in the liver parenchyma, a consequence of an activation of hepatic stellate cells (HSC) into myofibroblasts. Today, HSC-derived myofibroblasts are regarded as the most downstream cellular effectors of liver fibrosis and thus the primary target for the development of new antifibrotic therapies.
Mesenchymal stem cells transplantation is currently positioned as a promising emerging perspective for the treatment of liver fibrosis. Those cells have been proposed based on their hepatocytic differentiation, regeneration potential as well as their immunomodulatory properties. Our data demonstrated that adult liver mesenchymal stem cells efficiently inhibited HSC proliferation and collagen secretion in vitro, mainly via HGF. Increased secretion of other antifibrotic factors by treated HSCs was also noted. In vivo, we showed that repeated intrahepatic transplantation of our liver cells was correlated with a decrease in the expression of markers related to liver fibrosis.

Is-39: Enhanced Cell Substrate Impedance Sensing for Neuronal Differentiation Monitoring

Sanati Nezhad A

Department of Mechanical and Manufacturing Engineering, BioMEMS and Bioinspired Microfluidic Laboratory, Calgary Centre

for Innovative Technology (CCIT), University Of Calgary, Alberta, Canada
Email: amir.sanatinezhad@ucalgary.ca

Is-40: Nanoscale Optoregulation of Neural Stem Cell Differentiation by Intracellular Alteration of Redox Balance

Sanati Nezhad A

Department of Mechanical and Manufacturing Engineering, BioMEMS and Bioinspired Microfluidic Laboratory, Calgary Centre for Innovative Technology (CCIT), University Of Calgary, Calgary, Alberta, Canada
Email: amir.sanatinezhad@ucalgary.ca

Is-41: Molecular and Functional Characterisation of Vessel Resident Human Endothelial Progenitor Cells

Shafiee A[1, 2], Patel J[1], Fisk NM[1], Khosrotehrani K[1, 3*]

1. UQ Centre for Clinical Research, The University of Queensland, Brisbane, Australia
2. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
3. UQ Diamantina Institute, The University of Queensland, Brisbane, Australia
Email: k.khosrotehrani@uq.edu.au

Background: Although endothelial progenitor cells (EPC) have long been described, significant controversy around their identity remained. In the present study, we undertook to systematically test different cell populations from human placenta upon cell sorting.
Methods and Results: Upon magnetic sorting based on CD45 and CD34, we found that most endothelial colony forming potential was concentrated in the CD45-CD34+. Upon further flow sorting of CD45-CD34+ cells, three populations were observed based on CD31 level. Single cell culture assays were performed on CD31negative, CD31int and CD31hi. Only the CD31int cells were able to grow high proliferative potential EPC. CD31neg population contained mesenchymal cells whereas CD31hi cells only produced mature endothelial clusters. RNA sequencing of these populations identified Notch signalling as a key driver of endothelial progenitors as opposed to the mesenchymal subtypes. When the CD31int population was further characterised it was established that EPC are vessel resident but not in direct contact with the circulation as confirmed by flow cytometry and immunostaining. We next looked further at a functional level at the selfrenewing fraction of the endothelial colonies *in vitro* and established an IL33-P57 pathway maintaining progenitor quiescence and selfrenewal that was dependent on Notch signalling. This was validated *in vitro* and *in vivo* by performing shRNA and pharmacological inhibition of the different elements of this pathway.
Conclusion: Overall, our study uncovers a population of EPC *in vivo* in human term placenta at the cellular and molecular level and identified a key and novel role for Notch signalling in maintaining progenitor function *in vivo* and *in vitro*.

Is-42: Functional Definition of Endothelial Hierarchy from Progenitor to Mature Endothelial Cells in Adult Vasculature

Patel J[1], Seppanen EJ[1], Rodero MP[1], Wong HY[1], Donovan P[2], Fisk NM[1], Francois M[3], Khosrotehrani K[1, 2*]

1. UQ Centre for Clinical Research, Experimental Dermatology Group, The University of Queensland, Brisbane, Australia
2. UQ Diamantina Institute, Translational Research Institute, The University of Queensland, Woolloongabba, Australia
3. Institutea of Molecular Biosciences, The University of Queensland, Brisbane, Australia
Email: k.khosrotehrani@uq.edu.au

The existence of endothelial progenitors and their contribution to vasculature in adults has been controversial. Our aim was to define vesselresident endothelial progenitors *in vivo*.
Using common endothelial markers (CD34, CD31, VEGFR2) with flow cytometry in Cdh-5cre^{ERt2}/Rosa-YFP reporter mice, three subpopulations of endothelial cells could be identified among YFP+ and Lin cells. These were termed endovascular progenitor (EVP, CD34+CD31loVEGFR2lo), transit amplifying (TA, CD34loCD31intVEGFR2lo) and differentiated (D, CD34+CD31hiVEGFR2hi). Only EVP cells and not TA and D cells had selfrenewal capacity as demonstrated by *in vitro* colony forming assays and *in vivo* transplant studies in MatrigelTM plugs in recipient mice. Importantly, EVP cells arose from vascular resident beds that could not be transferred by bone marrow transplantation. In lineage tracing studies in wounds, EVP cells gave rise to TA and D cells. RNA sequencing on flow sorted populations revealed that EVP cells highly expressed genes related to progenitor function such as Sox9, Il33 whereas D cells highly expressed genes related to differentiated endothelium such as Cd31, Vwf and Notch. Sox18 transcription factor had a significant role in EVP to D differentiation, as determined by lineagetracing using Sox18CreERt2/Rosa-YFP mice. In the absence of functional SOX18/SOXF, EVP progenitors were still present, but TA and D populations were significantly reduced.
EVP cells is a functionally and molecularly defined resident progenitor *in vivo*. This is a paradigm shift in our understanding of vascular resident endothelial progenitors in adult endothelial regeneration.

Is-43: DNA Methylation Maintenance in Mouse Preimplantation Development

Walter J

Department of Genetics and Epigenetics, Saarland University, Campus Saarbrücken, Saarbrücken, Germany
Email: j.walter@mx.uni-saarland.de

In mammals genome wide DNA methylation is extensively reduced during the first cleavage stages of preimplantation development. It remains to be clarified to which extent and at which stages mechanisms of active/passive DNA demethylation are contributing to this loss of DNAmethylation. We applied high-resolution hairpin bisulfite sequencing to precisely follow the fate of symmetrical DNA methylation across the first cell divisions. We observe that a major proportion of chromosomes acquires a hemimethylated status after the completion of the first DNA replication. This indicates an impaired methylation maintenance as the major source of DNA-demethylation. Still a substantial proportion of sequences remain fully methylated on

both DNA strands showing that maintenancemethylation is not totally impaired. In the following cleavage stages DNA-methylation levels remain constant and a second significant decrease of DNA-methylation occurs at 3, 5dpc. During all preimplantation stages chromosomes exhibit a high level of mosaic hemimethylated CpGs. This points towards a dynamic fluctuation of DNA-methylation caused by a combination of passive demethylation, presumably caused by oxidized forms of 5-methylcytosine, and maintenance or de novo methylation activities.

Is-44: Epigenomics of Stem Cells

Walter J

Department of Genetics and Epigenetics, Saarland University, Campus Saarbrücken, Saarbrücken , Germany
Email: j.walter@mx.uni-saarland.de

Epigenetics is a key discipline for understanding processes controlling cellular reprogramming, differentiation and aging. Epigenetic control involves multiple molecular levels including various forms of DNA-methylation, multiple histone modifications and numerous forms of noncoding RNAs. The combination of these layers transforms the unique genome of an organism into hundreds of different cell type specific epigenomes that originate in the process of development and differentiation. The understanding of the epigenetic landscape of individual cells will greatly contribute to our understanding of cell type specific gene regulation in health and disease. In my lecture I will discus the Next generation sequencing based generation and interpretation of epigenome datasets as performed by the International Human Epigenome Consortium IHEC and in particular the German Epigenome Programme DEEP. In my talk I will introduce in the main technologies, their application and interpretation levels.

Oral Presentations

Os-1: CRISPR Mediated Knock-In Mouse Models for *In Vivo* Neural Reprogramming and Lineage Tracing

Basiri M, Shahbazi E, Asgari B, Moradmand A, Fallahi M, Tahamtani Y, Baharvand H

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
Email: mohsenbasiri@gmail.com

In vivo genetic stimulation and tracing of cell fate conversion provide worthy insights into mechanisms involved in cellular reprograming and tissue regeneration. In this study, we applied CRISPR technology to generate two knock-in mouse models for *in vivo* neural reprograming and in vivo lineage tracing. We have previously shown that forced expression of Zfp521 gene promotes the reprogramming of mouse and human fibroblasts into neural stem cells (NSCs). Using CRISPR technology we generated knock-in mouse embryonic cell (mESC) line harboring an inducible Zfp521-expressing cassette in ROSA26 locus. Knock-in ROSA26Zfp521 mouse model were generated through blastocyst injection of mESCs. Fibroblasts derived from homozygous ROSA26Zfp521/Zfp521 mouse models into NSCs after doxycycline treatment. In order to generate the second mouse model for lineage tracing, we inserted a conditional two color fluorescent protein expressing cassette into ROSA26 locus. This cassette contains a floxed membrane red fluorescent protein (mRFP) gene and a membrane green fluorescent protein (mGFP). Cre mediated excision of mRFP gene results in mGFP expression leading to a fluorescent color switching. Resulted ROSA26mRmG mice were mated with Tg(CreERT) mice and fibroblasts derived from double transgene pups were shown to be able to switch the fluorescent color in response to tamoxifen treatment. These knock-in mice can be applied for in vivo modeling and investigating transdifferentiation and regeneration phenomena.

Os-2: Production of Knockout Mice with FAM83H Gene Modifications by CRISPR/Cas9 Mediated Genome Engineering

Fathi F*, Parsa S, Naseri Sh, Soleimani F, Bahrami S, Banafshi O, Ebadifar A, Bagher Khadem Erfan M, Rostamzadeh J, Rahimi K

Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: farfath@gmail.com

Fam83h gene encodes a nonsecreted protein known as Fam83h which is targeted for the nucleus and it predicted to play a role in the structural development and calcification of tooth enamel. In humans, Defects in gene FAM83H cause autosomal dominant hypocalcified amylogenesis imperfecta (ADHCAI). In this study we applied CRISPR/Cas9 system to target Fam83h gene in mice. We directly microinjected the Cas9D10A mRNA (100ng/µl) mixed with sgRNAs (50 ng/µl) into cytoplasm. Then two cell embryos Transferred to 0.5 day psudopregnant mouse and pups were born after 19 days. In order to screen newborn pups DNA extraction was performed from tail biopsies and PCR was conducted using specific primer for targeted region of Fam83h. Target region for FAM83H gene disruption by Crisper-Cas9mediated cleavage were identified by DNA sequenceing.Our results showed that after Microinjection of mixed cas9/sgRNA into 135 zygotes, 75 zygotes were survived, 55 zygotes were developed to 2cell stage and then transferred to 0.5 day psudopregnant mice. 11 Pups were born and results of sequencing analysis showed that 7 mice have mutations in interested region. Among 7 knockout mice, 6 mice (85.7%) have deletion, 5 mice (71.4%) have nucleotide substitution, 3 mice (42.8%) have insertion along aimed genomic location . analysis phenotype of mutant mice showed the Mice carrying mutation in targeted region, in compare with control mice have small size and scruffy coat phenotype and one of them have defect in tooth formation.

Keywords: Amelogenesis Imperfecta, Hair Defects, Knockout Mouse, FAM83H Gene, Truncation Mutation

Os-3: Predicting The Concentration of Trace Elements Using Solvent Bar Microextraction HPLC-UV Technique Coupled with Adaptive Network Based Fuzzy Inference System

Gholghasemi M[1*], Qomi M[2]

1. Department of Medicinal chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Pharmaceutical Ingredients Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
Email: marjan.gholghasmi@gmail.com

Background: The aim of this study was to validate a method for predicting the concentration of critical compounds in complicated matrices such as cell cultures and to avoid extra costs. Solvent bar microextraction technique is a sample preparation method prior to highperformance liquid chromatography-Ultra violet analysis for complicated matrices such as urine, blood, stem cell culture, and waste water. This method, when coupled with adaptive network based fuzzy inference system, can detect and predict the concentration of trace elements and drugs at ultratrace levels in complicated matrices.
Materials and Methods: Ropinirole was used as a model drug for validation of this method. Therefore, six parameters(pH of donor and acceptor phase, stirring rate, time, temperature, and salt addition) affecting the preconcentration and determination of this drug were investigated. In this method, pH gradient was applied to transfer the drug into the solvent bar. The experiment was designed using Design-Expert version 7.0.0. 27 experiments were carried out, accordingly. MATLAB version 2010 was used for data analysis. Construction of an input-output mapping was done based on the results obtained from the experiments. For the simulation, the ANFIS architecture was employed to model nonlinear functions, identify nonlinear components in a control system, and predict a chaotic time series, all yielding remarkable results. Based on the best model chosen, the drug was preconcentrated and analyzed under the optimum condition.
Results: The limit of detection, limit of quantification, and preconcentration factor were the best result ever obtained. As a result, this method can be employed for preconcentration and microextraction of several elements, drugs, antibodies at trace levels in complicated matrices. After modeling, the optimum

condition could be predicted without performing unnecessary and expensive experiments.
Conclusion: Antibodies and Certain biomarkers can also be preconcentrated and detected using the proposed method. It offers high sample clean up, therefore it can be used for clean validation. Prediction of the course of treatment may be possible with the proposed method, therefore it is highly practical, easy and cost effective.
Keywords: Solvent Bar Microextraction, Adaptive Network Based Fuzzy Inference System, Chemometrics, Cell Culture

Os-4: 3D Bioprinting and Synchrotron Based In Situ Assessment of Cell Embedded Tissue Engineered Constructs

Izadifar Z

Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Canada
Email: Izadifar@lunenfeld.ca

Tissue engineering has been emerging as a promising approach to promote regeneration of functional tissues for repairing damaged tissues and organs. Hybrid engineered constructs fabricated from cells, hydrogels, and solid polymeric materials provide essential biological, structural, and mechanical features for natural like tissue regeneration. However, fabrication of customized hybrid constructs with embedded cells is challenging using conventional methods. Novel biofabrication methods can be developed using advanced technologies, such as 3D printing, to create designed tissue constructs from multiple synthetic and cell embedded biomaterials. Furthermore, it is noticed that nowadays evaluating the success of tissue engineered constructs largely relies on invasive techniques in which animal models must be sacrificed for histological analysis. This becomes a critical issue as tissue engineering advances from animal studies to human trials. Noninvasive methods based on novel synchrotron imaging techniques can be developed to assess engineered constructs in situ.
In this research, a 3D hybrid bioprinting fabrication method was developed to make 3D tissue constructs from polycaprolactone solid polymer, alginate hydrogel, and different type of cells for cartilage tissue engineering. Furthermore, novel in situ assessment methods were developed based on computed tomography (CT) synchrotron phasebased X-ray imaging techniques to examine soft tissue constructs implanted in pig knee cartilage in situ. The 3D bioprinting method was successfully developed and the embedded cells showed high level of biological performance; cell viability, proliferation and cartilage differentiation, in the 3D bioprinted hybrid constructs. The results demonstrated the promise of the developed method for cartilage and other tissue engineering applications. The developed phasebased assessment methods successfully visualized the tissue constructs with detailed structural properties in situ (not visible with clinical CT and MRI). The noninvasive assessment methods demonstrated promising for providing information and means to track the success of engineered constructs in tissue repair and remodeling in live animal and eventually in human patients.

Os-5: Non Invasive Approaches for Screening, Diagnosis and Monitoring of Neurodegeneration

Khaligh Razavi SM

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, Cell Science Research Center, ACECR, Tehran, Iran
Email: s.mahdirazavi@gmail.com

Neurodegenerative disorders, including dementias and Alzheimer's disease, continue to represent a major economic, social and healthcare burden. These diseases remain underdiagnosed or are diagnosed too late for meaningful interventions. The development of screening tests capable of detecting neurodegenerative diseases during early, preferably asymptomatic, stages has been a highly unmet need. Since such tests will be used for screening large populations of people, they should be noninvasive, inexpensive, and ideally independent of language and education. We have developed a 5 minute integrated cognitive assessment tool (ICA) that meets these criteria, and can be additionally used for monitoring the progression of the disease or effectiveness of the treatments.

Os-6: RNA Epitranscriptome and A Brand New Class of Biomarkers for Basic and Clinical Research

Khoddami V

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: vahidkhoddami@yahoo.com

Os-7: CRISPR/Cas9-Mediated Somatic Correction of A Novel Coagulator Factor IX Gene Mutation Ameliorates Hemophilia in Mouse

Liu Mingyao[1, 2], Zhenliang S[3], Honghui H[4], Yuting G[1], Ning M[5], Yanhong Y[6], Yuanhua H[5], Dali L1, Qi L[5], Yanlin M[5, 6*]

1. Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, China
2. Fengxian Hospital, Southern Medical University, , Shanghai, China
3. Bioray Laboratories Inc., Bioray Laboratories Inc., Shanghai, China
4. Department of Molecular and Cellular Medicine, Institute of Biosciences and Technology, Texas A and M University Health Science Center, Houston, United States
5. Hainan Provincial Key Laboratory for Human Reproductive Medicine and Genetic Research, Hainan Reproductive Medical Center, the Affiliated Hospital of Hainan Medical University Hainan Medical University, Haikou, China
6. Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, , Guangzhou, China
Email: 1742277533@qq.com

Background: Hemophilia B (HB), an Xlinked genetic bleeding disorder caused by deficiency of coagulator factor IX (FIX), affects 1 of every 25, 000 to 30, 000 males worldwide. As solely increasing the plasma FIX levels as low as 1% results in significant restoration of clotting activity, HB is considered a good model for evaluating the efficacy of distinct gene therapy strategies. Recent studies, such as the way of zinc finger nuclease, demonstrated that sitespecific integration of human F9 cDNA into transcriptionally highly active genomic loci in hepatocytes. However, direct correction of the F9 mutation by targeting the endogenous locus is to restore the clotting activity through so-

matic genome editing is still not adopted. Pioneer studies have shown a mouse model of genetic disease caused by a point mutation was phenotypically restored via Cas9 mediated gene repair *in vivo*. Based on these facts, our study is to confirm the phenotype of a novel mutation in factor IX gene and explore whether CRISPR/Cas9 mediated somatic correction of the mutation can ameliorate hemophilia in mouse.

Materials and Methods: Blood DNA was extracted and sequenced from the proband and other relatives to identify a novel mutation causing disease. And the mouse model of hemophilia B was generated by coinjection of Cas9 mRNA, sgRNA, and ssODNs carrying corresponding mutations. Adenoviral vectors containing the donor template and the sgRNA were delivered into adult mice by tail vein injection. We subsequently test the Activated Partial Thromboplastin Time (aPTT) and prothrombin time (PT).

Results: We identify a family with hemophilia B carrying a novel mutation, Y371D, in the human F9 gene (position 381 in mouse FIX), dramatically disrupting FIX activity. We used the CRISPR/Cas9 system to generate distinct genetically modified mouse models and confirmed that the novel Y371D mutation resulted in a more severe hemophilia B phenotype than the previously identified Y371S mutation. We subsequently deliver Cas9 components targeting the F9 Y371D mutation in adult mice. And test of clotting activity by aPTT and PT indicated HB in F9 mutant mice was ameliorated through naked DNA injection of Cas9 components. Our results show correction of 0.56% of endogenous F9 alleles in hepatocytes, which was sufficient to restore hemostasis in hemophilia B mice.

Conclusion: Our studies suggest that CRISPR/Casmediated in situ genome editing could be a feasible therapeutic strategy for human hereditary diseases, although an efficient and clinically relevant delivery system is required for further clinical studies.

Keywords: Gene Therapy, Genome Editing, Hemophilia B, Hemostasis, Monogenetic Disease

Os-8: Attitude of Researchers toward The Future Trend of Stem Cell Researches

Lotfipanah M[1, 2, 3*], Totonchi M[4], Azadeh F[5], Omani Samani R[2]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

2. Department of Medical Library and Information Science, Faculty of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran

3. Medical Library and Information Sciences Department, The School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran

4. Department of Medical Library and Information Science, Virtual School, Center for Excellence in E Learning in Medical Education, Tehran University of Medical Sciences, Tehran, Iran

5. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Email: lotfipanah_setad@yahoo.com

Background: Stem cells with unlimited proliferation potentialities as well as differentiation potency are considered as a promising treatment method for incurable diseases in future. Among all types of stem cells and different organs and systems the trend of researches has undergone modifications. There is always a question that how these researches will be directed in the future? The aim of the present study is to evaluate the future trend of stem cell researches from researcher's view points.

Materials and Methods: This was a cross sectional descriptive study on researchers involved in stem cells in Royan Institute. A questionnaire was designed using qualitative study on expert opinion and review literature. Content validity was done using three rounds of Delphi method with 18 experts. Face validity was done by a Persian literature expert and a graphics designer. The questionnaire was distributed among 150 researchers involved in stem cell studies engaging in Royan Institute biology labs.

Results: 138 complete questionnaires were collected. Mean age of participant was 5.8 ± 31.13 most of whom (60.9%) were females. Budget was considered as the most important issue in stem cell research (76.1% of participants) needing governmental financial support (79.7%), but charities can contribute substantially to this project (77.5%). 90.6% of participants stated that stem cells should lead to commercial usage which can support the future researches in itself (86.2%). The aim of stem cell research was stipulated as increasing health status of the society (92.8% of the participants). at present, among cell types, the importance has been attached to cord blood and adult stem cells. Researchers pin importance on mesenchymal stem cells rather than hematopoietic stem cells (57.73%). The prime priorities were given to cancer so that stem cell research can be directed to the sphere stem cell research. But the least preference was given to skin researches. It is argued that international collaboration will pave the way for promotion of stem cell researches.

Conclusion: Regenerative medicine is considered as future of stem cell research with emphasis on application of these cells especially in cancer treatments

Keywords: Stem Cell, Regenerative Medicine, Treatment, Future Study, Attitudes

Os-8:Mesenchymal Stem Cells Conditioned Medium Therapy Modulates Spinal Neuroinflammatory Symptoms

Nazemian V[1, 2*], Zaringhalam J[2], Manaheji H[2], Sharifi AM[3]

1. Neurophysiology Research Center, Medical Faculty, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Department of Physiology, Medical Faculty, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3. Tissue Engineering Group, Department of Orthopedic Surgery, University of Malaya, Kualalampur, Malaysia

Email: vida.nazemian@gmail.com

Background: Interest in neuroinflammation and neuroimmune activation has grown rapidly in recent years with the recognition of the role of central nervous system (CNS) inflammation and immune responses in the etiology of neurodegenerative disorders such as rheumatoid arthritis (RA). Proinflammatory cytokines and chemokines have been strongly implicated in the generation of pathological pain states at both peripheral and CNS sites. Constitutive overproduction of inflammatory cytokines, such as interleukin 1beta (IL-1β) in inflammated cells, has been indicated to play a pivotal role in the RA pathogenesis which is accompanied with inflammatory symptoms such as edema, hyperalgesia and pain. One area that has emerged as promising therapeutic targets for the treatment of RA and alleviation of spinal neuroinflammatory symptoms is the modulation of CNS immunological responses. Mesenchymal stem cells conditioned medium (MSC-CM) has antiinflammatory factors which can adjust the immune responses and has a paracrine effects as well. The aim of this study was to investigate

the effect of administration of MSC-CM on spinal neuroinflammatory symptoms.
Materials and Methods: Complete Freund's adjuvant (CFA)-induced arthritis was caused by single injection of CFA into the rats' hind paw on day 0 (s.c.). MSC-CM was administered daily after CFA injection (i.p.). Hyperalgesia, Edema, Serum IL-1β variations were assessed on days 0, 7, 14 and 21 of the study.
Results: The results of this study implicated the role of MSC-CM in alleviating spinal neuroinflammatory symptoms such as edema and hyperalgesia and decreasing the serum levels of IL-1β during different phases of neuroinflammation caused by CFA.
Conclusion: It seems that MSC-CM treatment due to its direct effects on inhibition of IL-1β as a proinflammatory cytokine can alleviate neuroinflammatory symptoms during CFA induced arthritis.
Keywords: Neuroinflammation, Rheumatoid Arthritis, Hyperalgesia, Edema, IL-1β

Os-10: The Peptidyl Prolyl Isomerase PIN1 Upregu Lates Nrf2 Expression in H-Ras Transformed Mammary Epithelial Cells

Saeidi S[1, 2*], Kim SJ[1, 2], Han HJ[1, 2], Piao JY[1, 2], Surh YJ[1, 2]

1. Tumor Microenvironment Global Core Research Center, College of Pharmacy, Seoul National University, Seoul, South Korea
2. Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, and College of Medicine or College of Pharmacy, Seoul National University, Seoul, South Korea
Email: soma_saeidi@yahoo.com

Background: The nuclear factor erythroid 2 (NFE2) related factor 2 (Nrf2) is a transcription factor that integrates cellular stress signals and rescues cells from a wide range of noxious stimuli. However, recent studies have revealed the constitutive overexpression of Nrf2 in many transformed or cancerous cell lines and human tumor tissues, which may confer an advantage for survival and growth of cancer cells. PIN1, a peptidyl prolyl isomerase, was originally discovered in searching for mitosis associated molecules, and it is overexpressed in many types of malignancies including breast, prostate, lung and colon cancers. PIN1 specifically recognizes phosphorylated serine (Ser) or threonine (Thr) immediately preceding a proline (Pro) residue (pSer/Thr-Pro) and isomerizes the peptide bond. We have speculated that Nrf2 harbouring the pSer/Thr-Pro motif provides a binding site for PIN1. In this study, we found that the levels of Nrf2 and PIN1 and their mRNA transcripts were elevated in rastransformed human breast epithelial (MCF10A-ras) cells compared to those in nononcogenic MCF10A cells. PIN1 silencing with shRNA considerably reduced the expression of Nrf2 in nucleus without influencing its mRNA level in the MCF10A-ras cells. PIN1 silencing also led to downregulation of heme oxygenase-1, a major downstream target of Nrf2. In contrast, knockdown of Nrf2 with siR-NA did not have significant effect on PIN1 expression. Notably, Nrf2 and PIN1 physically interacted each other in nucleus, but there was interaction between PIN1 and Keap1 in cytosol, a negative regulator of Nrf2. Taken together, these findings suggest that PIN1 stabilizes Nrf2 through direct interaction with this transcription factor.
Materials and Methods: Pin1 and Nrf2 protein expression was examined by immunofluorescence in Hras MC-F10A cells. Colocalization of Pin1 and Keap1 assessed by immunofluorescence staining in Hras MCF10A cells. The mRNA and protein levels of Nrf2 and Pin1 were determined by RT-PCR and Western blot analysis, respectively in Hras MCF10A cells. The protein levels of HO-1 and Pin1 were examined after transfection with Nrf2-siR-NA or Pin1-siRNA . Protein lysates of Hras MCF10A cells were immunoprecipitated with Keap1, Nrf2 and Pin1 antibodies, and the proteins were detected with same antibodies and vice versa.
Results: Keap1-Nrf2 axis is interacts with Pin1, and this interaction is momentous for the transcriptional activity of Nrf2.
Conclusion: Pin1 interacts directly with Keap1 in cytosol Pin1 may changes the conformation of Keap1 by binding to its WW domain Nrf2 appears to dissociate from Keap1 after the interaction of Pin1-Keap1 Pin1 directly interacts with Nrf2 only in nucleus.
Keywords: Pin1, Nrf2, Kea

Os-11: Tau Is The Leading Cause of Neural Cell Death Upon Stress Conditions

Shahbazi S[1, 2*], Shahpasand K[2]

1. Department of Cell and Molecular Biology, Faculty of Biology, University of Tehran, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: shahpasand09@gmail.com

Background: Natively unfolded tau is a microtubule associated protein (MAP) predominantly expressed in neurons, which stabilizes microtubules and promotes their assembly. However, it becomes abnormally hyperphosphorylated insoluble and filamentous in a number of neurodegenerative disorders collectively known as tauopathies. The biological functions of this protein can be regulated through phosphorylation or post phosphorylation modifications. It has been reported that phosphorylated tau at Thr231(pT231-tau) could be exist in two distinct cis and trans conformations in which cis displays both toxic gainsof function and loss of normal function and leads to neuronal tauopathy *in vitro* and *in vivo*. Studies indicated that tau is also present at low levels in normal glial cells; however, only trace amounts of tau can be detected in normal human and rodent astrocytes and does not play much roles in astrocytic cytoskeletal element. We herein have designed *in vitro* experiments to demonstrate ptau production is both necessary and sufficient for cell death induction.
Materials and Methods: Cortical primary neurons were isolated from 16 day old embryonic mouse brain and seeded on polyL-lysine coated dishes and then subjected to hypoxia treatment for 48 h, followed by live and dead cell assay Primary mouse astrocytes were prepared using P1 to P4 mouse pups and were co transfected with GFP–tau, p25, Cdk5 and EGFP.
Results: Hypoxia treatment induced cell death in primary neurons but not primary glial cells. Overexpression of human tau with p25, Cdk5 led to cell death in primary mouse astrocytes.
Conclusion: Our results clearly demonstrate the fact that hyperphosphorylated tau is both necessary and enough for cell death induction and would help us find an efficient therapeutic strategy against tauopathies.
Keywords: Tauopathy , Glial Cells, Hyperphosphorylated Tau

Os-12: Cis pT231tau Drives Neurodegeneration in Bipolar I Disorder Studied Through Cellular Models

Shahpasand K, Naserkhaki R*

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: shahpasand09@gmail.com

Background: Bipolar disorder is a complex neuropsychiatric disease that is characterized by intermittent episodes of mania and depression in which abolished treatment would result in 15% committed suicide in the patients. Recent studies indicate that tauopathy may have contribution in pathogenesis of bipolar disorders. For example, blocked GSK3β employing lithium and also increased SFPQ expression which interrupts tau gene splicing upon bipolar disorder demonstrate of possible tauopathy mediatory role in the disease. Furthermore, argyrophilic grains composed of phosphorylated tau have been observed in postmortem brains of bipolar patients. On the other hand, recent studies have demonstrated that phosphorylated tau at Thr231 exists in two distinct cis and trans conformation in which cis pT231tau 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 GSK3β in SH-SY5Y cells and examined cis ptau and total tau in these models by immunofluorescence and western blotting.
Results: We have found that cis ptau increased in mania model of bipolar disorders showing that it may contribute to pathophysiology of bipolar disorders and could be the cause of neural cell death upon the disease.
Conclusion: Our findings demonstrate the molecular mechanism of neurodegeneration upon bipolar disorder which in turn would suggest novel therapeutic strategies against the disease.
Keywords: Bipolar Disorders, Cistauosis, GSK3-β, Tauopathy

Os-12: Erk1/2 Pathway Regulates The Erythroid Differentiation of Cord Blood Hematopoietic Mononuclear Cells

Zarrabi M[1, 2], Afzal E[2*], Asghari Vosta Kola MH[1], Ebrahimi M[1]

1. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mebrahimi@royaninstitute.org

Background: Human umbilical cord blood (hUCB) is the best source of hematopoietic stem and progenitor cells (HSCs/PCs) for transplantation. So far, extensive efforts have been made to identify the mechanisms that control the selfrenewal and differentiation of HSCs/PCs. ERK1/2 pathway plays an important role in proliferation, differentiation and survival of animal cells. The aim of this study was to understand the role of MEK/ERK pathway in *ex vivo* selfrenewal and differentiation of nonpurified mononuclear cells (MNCs).
Materials and Methods: In this study, UCB mononuclear cells were cultured for 10 days in serum free liquid cultures containing stem cell factor (SCF), FLT3 ligand (FL), thrombopoietin (TPO). We used a specific MEK inhibitor PD0325901 (PD) to block the ERK pathway. Cell counting, immunofluorescence staining, colony assay and realtime analysis were used to evaluate the effect of ERK inhibition on UCB-derived cells.
Results: Here we report that although culture the cells in the presence of PD, significantly reduced the expansion of CD34+ cells and CD34+ CD38 cells, the expression of stemness related genes did not change. Moreover, PDMNCs cells showed less ability in formation of granulocyte monocyte (GM) and granulocyte erythrocyte macrophage megakaryocyte (GEMM) colonies. Interestingly, when ERK pathway was blocked in UCBMNCs, erythroid differentiation was promoted which was in concomitant with increasing the number of burstforming uniterythroid (BFU-E) colony, upregulation of some erythroid differentiation related genes as well as down regulation of erythroid differentiation repressor genes. The increase in the expression of erythroid surface marker glycophorin A (CD235) confirmed our data for erythroid differentiation of UCB-MNCs.
Conclusion: Taken together, our results indicate that MEK/ERK pathway has a critical role in the UCB-MNCs selfrenewal and differentiation *ex vivo*. Also, we suggest that MEK/ERK inhibition likely could be a new key for erythroid induction of hematopoietic mononuclear cells.
Keywords: MEK/ERK Pathway, Erythroid Differentiation, Human Umbilical Cord Blood, Hematopoietic Mononuclear Cells

Poster Presentations

Ps-1: The Apoptotic Effect of Microvesicles Derived from Mesenchymal Stem Cells on The NB4 Cells

Abbaszade Dibavar M*, Soleimani M

Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: m.soleim@modares.ac.ir

Background: Stem cell based therapy as a novel approach is propounded for treatment of autoimmune and other hematological diseases and also used in regenerative medicine. There has been a significant growing interest in stem cells and induced pluripotent stem (iPS) cells' utilization, especially mesenchymal stromal cells (MSCs) in combination with tissue engineering in recent years. The use of MSCs as a therapeutic option shows a promising future due to their immunoprivileged status and immunomodulatory properties. MSCs are plastic adherent nonhematopoietic multipotent cells that have the ability of renewal and differentiation into cells. there is a need for effective treatments to address chemo resistance in the APL. Recent studies considered microvesicles as a potential therapeutic agent. Microvesicles are small membranebound particles released by different cells including healty and tumor types. Microvesicles can transfer their contents, proteins and RNAs, to target cells and thereby transform them. This may induce apoptosis or survival depending on cell origin and the target cell. As point of view, microenvironment of bone marrow, normal and leukemic cells have interchangeable interaction through microvesicles, so microvesicles derived from human bone marrow mesenchymal stem cells might affect leukemic cells. In this study, we investigated the apoptotic effect of microvesicles derived from mesenchymal stem cells on NB4 cell line.
Materials and Methods: MSCs were cultured in culture medium. Microvesicles were isolated from Mesenchymal stem cells by ultra centrifugation and were added to NB4 cell line. Also, NB4 cells without microvesicles were cultured as control group. After 7 days, cell count, cell viability By MTT assay and RT real time-PCR for BAX gene expression were performed.
Results: We demonstrate the effect of microvesicles derived from human bone marrow mesenchymal stem cells apoptosis of NB-4 cell line. This study shows, cell growth dramatically hindered and induction of apoptosis increased in NB4 cells, and BAX gene show significant rising.
Conclusion: Obtained results demonstrated that bone marrow microvesicles derived from human bone marrow mesenchymal stem cells have potential capability to play as effective therapeutic regimen in curing APL.
Keywords: Mesenchymal Stem Cells, Microvesicles, Apoptosis,

Ps-2: Conditions of The Legitimate Contracts of Stem Cell in Terms of Jurisprudence, Law and The Legal Views of Imam Khomeini

Ahmadkhanbeigi Kh*, Hasannia A, Mohaghegh Damad SM

1. Islamic Jurisprudence and Law, Imam Khomeini Institute, Tehran, Iran
2. Quran and Hadith Sciences, Shahed University, Tehran, Iran
3. The Islamic Bio Ethics Association, Tehran, Iran
Email: khanbaigi@yahoo.com

Background: Although production and use of stem cells has opened new horizons in medicine and treatment of incurable diseases, it has been accompanied with wide ethical and legal issues in the world. Researches play a vital role in the continued expansion of medical sciences and without fresh researches, new treatments still remain unclear. The therapeutic use of stem cells, although is applied after a long and accurate range of tests, dates back to a short time and its outcome is still unknown. In many cases, indeed therapeutic activities have research aspects, so the research contracts of therapy stem cells between a therapy center and a researcher must have eligible conditions stated in article 190 of Civil Code by the legislator and without any of these conditions the validity of the contract would be canceled. In therapeutic stem cells researches, first it must be checked whether the patient is able to make such a contract legally and juristically. Regarding the interdisciplinary studies of jurisprudence and law, the present paper is aimed to study and recover the relations between a human and his/her physical organs, that is disputed by many of jurists and lawmen. Some jurists and lawyers believe that a person dominates his/her body organs and it is in fact a rational issue, as if he/she possesses his/her property. Among them it must be mentioned of the late Ayatollah Khomeini and Ayatollah Makarem Shirazi. In contrast, others consider this relation as an inherent property: among them are the late Ayatollah Khou'ei, Ayatollah Asef Mohseni and Ayatollah Mohammad Mo'men. Seemingly, to have human trials research in accepting the risks of therapy is to the extent that some legal sentences must not be ignored. According to some scholars, “based on reason and law, any capture resulting in death, substantial and irreversible maim or harm, or to the desecration or humiliation of a human, is prohibited, inadmissible and unlawful”. Another application of stem cells is tissue and organ transplantation and the discussion of transaction of these organs; is a person allowed to capture his/her organs so that he/she can move them to others? In this context, the transfer of organs during the life of owner is permissible if he/she is safe of any physical harm, but if it causes to a harm which is not considered rationally, it is permissible.
Materials and Methods: In therapeutic stem cells researches, first it must be checked whether the patient is able to make such a contract legally and juristically. Regarding the interdisciplinary studies of jurisprudence and law, the present paper is aimed to study and recover the relations between a human and his/her physical organs, that is disputed by many of jurists and lawmen.
Results: In conclusion, in stem cell researches, as long as there are the requirements in terms of intellectual and religious domination of man over his body, a capture (to an organ) would be allowed as well. The forbidden and unlawful cases are those which result in death, substantial and irreversible maim or harm, or desecrate and humiliate a person. Therefore, in two areas of production and therapy application of stem cells, a human trial as long as does not violate lawful sentences, while capturing organs, is permitted to capture his/her selfbody organs.
Conclusion: In conclusion, in stem cell researches, as long as there are the requirements in terms of intellectual and religious domination of man over his body, a capture (to an organ) would be allowed as well. The forbidden and unlawful cases are those which result in death, substantial and irreversible maim or harm, or desecrate and humiliate a person. Therefore, in two areas of production and therapy application of stem cells, a human trial

as long as does not violate lawful sentences, while capturing organs, is permitted to capture his/her selfbody organs.
Keywords: Research Contracts, Stem Cells, No-Harm Rule, Tissue Transplant

Ps-3: Effect of Combination Therapy with Bone Marrow Stromal Cells (BMSCs), Triiodothyronine and Exercise on Cerebral Ischemia in Mice

Akhoundzadeh K[1*], Vakili A[1], Sameni HR[2], Safari M[2], Rashidy Pour A[1], Vafaei AA[1], Mohammadkhani R[1]

1. Department of Physiology, Research Center of Physiology, Semnan University of Medical Sciences, Semnan, Iran.
2. Department of Anatomy, Research Center of Neural Stem Cells, Semnan University of Medical Sciences, Semnan, Iran
Email: ab.vakili@yahoo.com

Background: Despite of extensive medical advancement, yet stroke treatment is an important issue in health care system. Since stem cells couldn't alone lead to satisfying results in stroke treatment, manipulated stem cells or combination therapies have been considered. This study was designed to explore effect of combination stem cell with thyroid hormone and exercise on stroke induced apoptosis and endogenous neurogenesis.
Materials and Methods: Cerebral ischemia, by Middle Cerebral Artery occlusion (MCAO), was induced for 45 minutes and reperfusion was allowed for 7 days in albino mice. Bone marrow stromal cells (BMSCs) were injected intracerebroventricularly 24h after ischemia. Mild exercise and T3 injection (20 µg/kg/daily S.C) were started 24h after MCAO and continued for 6 days. Animals were randomly divided into seven groups: sham, PBS (as control), BMSCs, TH, EX, BMSCs+TH, BMSCs+Ex and BMSCs+TH+Ex. Apoptosis (Tunnel positive cells) and neurogenesis (BrdU positive cells) were evaluated at 7th day after MCAO.
Results: Combination of stem cell transplantation along with exercise and thyroid hormone significantly reduced the number of TUNEL positive cells and increased BrdU positive cells. There was a significant difference between the BMSCs+EX+TH with other treatment groups except with BMSCs+EX and EX groups in BrdU positive cells ($P<0.001$). Also, there was significant differences between the BMSCs+EX+TH with other treatment groups ($P<0.01$) except with BMSCs+EX group in TUNEL-positive cells ($P>0.05$).
Conclusion: Our results suggested that TH and exercise could increase BMSCs efficacy in recovery of stroke in an experimental model of cerebral ischemia. Further perclinical and clinical studies are necessary to approve this strategy for treatment of stroke patients.
Keywords: Cerebral Ischemia, Exercise, Thyroid Hormone, Stem Cell Therapy

Ps-4: TNF-α Induction by Parvovirus B19 in Human Bone-Marrow Mesenchymal Stem Cells

Amiri Sh[1*], Atashi A[2], Gheibi N[1], Azad M[3], Elmi A[1], Ajami M[2], Taghavi Z[2], Rasaei N[2], Mozaffar M[2]

1. Department of Medical Biotechnology, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
2. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University of Tehran, Tehran, Iran
3. Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
Email: gheibi_n@yahoo.com

Background: Chronic inflammation plays an important role in tumor initiation, progression and invasion. Additionally, it has been proved by abundant data that the inflammation can be induced by many factors, including viral and bacterial infections. Parvovirus B19 (PVB19) is a common human pathogen which has notable tropism to human bonemarrow erythroid progenitor cells. However, it has shown that the virus can enter human bone marrow mesenchymal stem cells (hBM-MSCs) and persist in the infected cells lifelong. We hypothesized that the infection of hBM-MSCs as the fundamental cellular component of bone marrow niche by PVB19 may lead to secretion of pro-inflammatory cytokines.
Materials and Methods: hBM-MSCs were *in vitro* expanded in a standard medium and cultured up to passage three under standard conditions. The cells were then transfected with a plasmid containing B19 genome via Nucleofector. Total RNA was extracted from all studied groups 36 h subsequent to transfection, and the expression level of TNF-α was examined using qRT-PCR.
Results: Data analysis from qRT-PCR showed the significant increase in the expression of TNF-α gene in the cells which were transfected with B19V ($P<0.05$).
Conclusion: Until today, several researchers have indicated the persistence of B19V in a wide variety of tissues, especially bone marrow with active gene expression. Although more researches are required, our findings for the first time suggest the importance of B19V infection of hBM-MSCs to establish an inflammatory microenvironment in the bone marrow and its involvement in inflammation related diseases of hematopoietic organ. In conclusion, based on our results, molecular assay to diagnose B19V infection of hBM-MSCs prior to stem cell therapy is strongly recommended.
Keywords: Bone Marrow, Chronic Inflammation, Infection, Mesenchymal Stem Cell, Parvovirus B19

Ps-5: Effect of Esteradiol Treated Mesenchymal Stem Cell in Amiliorating Animal Model of Multiple Sclerosis

Asadi F[1*], Mirzaei MR[1, 2], Abtahi Froushani SM[3], Heidari Barchi Nezhad R[3]

1. Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
2. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
3. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: mirzaeemr@gmail.com

Background: Preliminary studies revealed that mesenchyal stem cells (MSCs) therapy is a worthwhile strategy to downregulate pathogenic immune responses in multiple sclerosis (MS). Nevertheless, insufficient implantation of cells to damaged brain and spinal cord has limited their potential therapeutic effects. There is evidence that estradiol (E2) enhances homing of stem cells. This study was done to investigate the therapeutic effects of E2 treated MSCs in experimental autoimmune encephalomyelitis as an animal model of MS.
Materials and Methods: EAE was induced in Wister rats by guinea pig spinal cord homogenates and complete Freund's adjuvant. Therapies were initiated at day 12post immunization

when the mice developed a disability score with 2×10^6 of E2 treated MSCs or MSCs without treatment. After day 33, the mice were sacrificed and the effects of cell therapy were investigated.
Results: Clinical scores, leukocyte infiltration and lymphocyte proliferation were significantly decreased in EAE mice received E2 treated MSC more prominent than EAE mice received MSCs without treatment. Furthermore, Body weight significantly improved in EAE mice received E2 treated MSC more prominent than EAE mice received MSCs without treatment.
Conclusion: Our finding indicates that conditioning of MSCs with E2 may be as a useful approach to control MS.
Keywords: Mesenchymal Stem Cell, Estradiol, Multiple Sclerosis, Experimental Autoimmune Encephalitis

Ps-6: IAP Gene Family Expression Profile in Tumor Cell Line, Bladder Cancer and Normal Tissue

Asadi F[1*], Mirzaei MR[2], Hassanshai GH[2], Mahmoodi M[2], Hajizadeh MR[2]

1. Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
2. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
Email: mirzaeemr@gmail.com

Background: Apoptosis were suppressed in cancer tissues and tumor cell lines because antiapoptosis genes were over expressed. Inhibitor of apoptosis proteins (IAP) gene family is one of the antiapoptotic gene families that control apoptosis pathway. The expression profile of eight genes from IAP family in bladder tumor cell line (5637), cancer and normal tissues of bladder cancer were the aim of this study.
Materials and Methods: In this experimental study, the 5637 tumor cell line was cultured in RPMI1640 medium. Cancer tissue samples were obtained from patients samples referred to the pathology laboratory. Tissue edges were selected as normal tissue. Expressional profile of interested genes was obtained by using specific primers and Real-Time PCR method.
Results: The results showed that the expression of seven of studied genes in tumor cell lines and cancer tissues were upregulated but BIRC4 (XIAP) gene was downregulation in both tumor cell line and tissues.
Conclusion: The expression of IAP gene family induced selfrenewal potency. The results showed that these genes were expressed in cancer tissue and cancer cell lines in compared to normal tissues. Data suggested over expression of antiapoptotic genes such as IAP family, trigger the cell to scope from apoptosis.
Keywords: IAP Gene Family, Tumor Cell Lines, Bladder Cancer

Ps-7: Valproic Acid Reverses The Epithelial to Mesenchymal Transition and Induces Apoptosis in Mir-302/367-Transfected Human Breast Cancer Cells

Atashkar N*, Javeri A, Fakhr Taha M

Department of Stem Cells and Regenerative Medicine, Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
Email: arashj@nigeb.ac.ir

Background: Reprogramming of cancer cells by epigenetic factors including miRNAs has recently been emerged as a new approach for tumor suppression. Embryonic specific miR-302 cluster has been shown to reprogram human breast, melanoma and colon cancer cells to a less invasive state and to reverse the epithelial to mesenchymal transition (EMT) process in these cells. Valproic acid has also been shown both as tumor suppressor and a reprogramming inducer in production of induced pluripotent stem cells. In this study, we aimed to investigate the effect of valproic acid on the EMT process in two invasive human breast cancer cells reprogrammed by overexpression of miR-302/367 cluster.
Materials and Methods: MDA-MB-231 and SK-BR-3 breast cancer cells were cultured and transfected by miR-302/367 cluster and a mock vector. Transfected cells were treated with 2 mM valproic acid every two days for a 2-week period. At the end of the culture period, different treatment groups were examined for morphological alteration, apoptotic rate by flow cytometry using Annexin V-PE/7AAd staining, and expression of some EMT markers by quantitative real-time PCR.
Results: The results showed more epithelial-like morphology in the cells transfected with miR-302/367 cluster which were also treated with valproic acid. The same treatment upregulated CDH1, downregulated VIM, CTN-NB1 and TWIST1 expression, and upregulated the early apoptotic rate significantly.
Conclusion: Generally, the results are indicating a prominent role for valproic acid in the reversal of EMT process and as an effective adjuvant for miR-302/367 in breast tumor suppression.
Keywords: miRNA, EMT, Valproic Acid, Reprogramming

Ps-8: Epigenetic Marks Comparison between Blood Cell Derived from iPSCs and ESCs

Azarafrouz F*, Farhangian M, Fallahi H

Department of Biology, School of Sciences, Razi Universitys, Kermanshah, Iran
Email: Fallahi.raziuni@gmail.com

Background: The epigenetic memory of a cell defines a set of modifications to the cell deoxyribonucleic acid (DNA) that do not alter the DNA sequence. They are inherited from the mother cell from which it descends to the progenies. Such modifications can alter gene expression and therefore the properties and behavior of the cell. Induced pluripotent stem cells (iPSCs) have been established from various somatic cell types. Accumulating evidence suggests that iPSCs from different cell sources have distinct molecular and functional properties similar to the embryonic stem cells (ESCs) including selfrenew by dividing and also can differentiate into any specialized cell of the body. It has been indicated that the source of somatic cells may be a major determinant of variation in the resulting iPSCs. Although, iPSCs can make almost any cells type, 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 looked at the dynamic of methylation marks on the DNA during conversion of iPSCs and ESCs into blood cells.
Materials and Methods: We have used previously published data to address the issue with differential capacity among different iPSCs. Briefly, iPSCs were generated from human dermal fibroblasts (HDFs), hematopoietic cells such as cord blood (CB) and peripheral blood (PB), dental pulp (DP) cells as well

as keratinocytes (Kara). we obtained differentially expressed (DE) genes. Next, methylation pattern of promoter regions of these DE genes were assessed. Then, differentially methylated regions (DMR) that are involved in defining differential capacity were extracted.
Results: Expectedly, 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 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 modelling or treatment.
Keywords: Epigenetic, iPSCs, ESCs, Methylation, Transcriptome

Ps-9: Methylation Variation between Reprogramming of Different Types of Somatic Cells into iPSCs

Azarafrouz F*, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: fallahi.raziuni@gmail.com

Background: Many studies shown epigenetic variation impacts on gene expression through histon methylation and DNA methylation. CpG islands are methylation region that influence the gene expression. One of the issues during conversion of somatic cells to iPSCs is the residual memory of somatic cells. Studies show that in reprogramming cells, differentiation methylation regions (DMRs), DNA methyltransferase enzymes (DNMTs): DNMT3A and DNMT3B methylated promoters are prime regulatory elements for up regulation or down regulation of gene expression. There is no direct report on the differnces in the methylation pattern of regulatory genes between somatic cells and their iPSCs counterparts. As such it is not clear if the quality of iPSCs derived from different somatic cells are due to residual memory or else, the DMR is a consequence of conversion. To address this issue, we have conducted this study using freely available datasets.
Materials and Methods: In this study, microarray data regarding conversion of somatic cells into iPSCs including fibroblast, keratinocyte and blood cells were analyzed. Following identification of differentially expressed genes, the methylation pattern of promoter regions for these genes were extracted from methylation chip data. Then these data were integrated into human genomic data to correlate the differential expression of genes to their differential methylation pattern in the promoter regions.
Results: Our analysis showed different methylation pattern in promoter regions during conversion of somatic cells into iPSCs. DMR both hypo and hyper methylation are iinvolved in determining the similarities between the resulting iPSCs to the ESCs. However, it appears that DMR alone is unable to expoalin the differnces between the somatic cells gene expression upon conversion to iP-SCs.
Conclusion: We have identified DMR among several iPSCs drived from different types of somatic cells. Gene expression profile of the resulting cells confirmed that these DMr are involved in the alteration of expression pattern. Our findings could help in identification of permanent residual somatic memory in the iPSCs and pave the way to develop protocols to remove them from the iPSCs inorder to obtain more similar and homogenous iPSCs population for stem cell Therapy.
Keywords: DMR, Reprogramming, Data Analysis, iPSCs, Epigenetic

Ps-10: Regulation of Stemness and Metastasis in Melanoma, Ovarian Cancer, Breast Cancer and Gastric Cancer by Common miRNAs

Azimi M[1, 2*], Ebrahimi M[2], Firoozi J[2], Totonchi M[3]

1. Department of Cellular and Molecular Biology, Faculty of Genetics, University of Science and Culture, Tehran, Iran
2. Department of Genetics, Center of Reproductive Biomedical Research, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mebrahimi@royaninstitute.org

Background: Cancer is a leading cause of death worldwide; it kills thousand people each year. Several factors are link to metastasis and high risk of cancer, including cancer stem cells that can be noted. Since the stemness and metastasis features can regulate by common regulators, therefore, finding these common regulators of both processes can be helpful for targeting them simultaneously and removing the tumor and their micrometastasis. In this study, by using data mining and bioinformatics approach, we found out 9 micro-RNAs regulate both metastatic and stemness process in four cancers.
Materials and Methods: We used literature review to identify most common stemness and metastasis miR-NA target genes and then, we chose mirtarbase software in order to obtain to key microRNAs and then measure the expression of the selected genes and miRNAs in these four cancers by qRT-PCR. The process of micro-RNA selection: 1. First of all, we put up the selected stemness and metastasis genes within the mirtarbase software and collected all miRNAs which target these genes. 2. Next, The micro RNA which targeted, metastasis and metastasis at the same time were elected. 3. A list of miRNAs which targeted the most number of genes was performed by R software 4. After that, based of criteria (each miRNA should targets at least 3 stemness genes and 2 metastasis genes) will be filtered and 5. Finally we used literature review and draw a conceptual image to confirm the role of these miRNAs in regulation of metastasis and stemness process in four cancer related.
Results: Based on literature review 5 miRNAs including: hsamiR-21, hsa-200c, hsa-10b, hsa-520cm has-373 and based on different sets of bioinformatics datas, four miR-NAs including: hsa-miR-34a, hsa-miR-30, hsa-335, hsa-304 can play a crucial role for regulation of stemness and metastase process.
Conclusion: This study explained that these candidate miRNAs can target 2 mentioned properties at the same timeand may be useful biomarkers at early stage of these four cancers.
Keywords: Cancer, Bioinformatics, miRNA, Metastasis, Stemness

Ps-11: The Effect of microRNA Biomarkers on Selfrenewal, Metastasis and Resistance to drug in Gastric Cancer: A Systematic Review

Azimi M[1, 2*], Ebrahimi M[3], Firoozi J[2], Totonchi M[3]

1. Department of Genetics, Center of Reproductive Biomedical Research Center, Tehran, Iran
2. Department of Cellular and Molecular Biology, Faculty of Genetics, University of Science and Culture, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mebrahimi@royaninstitute.org

Background: Cancer stem cells (CSCs) have been detected as rare cell populations in many cancers and play a critical role in tumor growth, resistance to drugs and metastasis. They are known as novel targets for antitumor strategies. Recent studies have highlighted the important role of miRNAs in controlling the selfrenewal, tumor onset, metastasis and chemoresistance in tumor cells. Therefore, the purpose of this study is to find out these multi functional miRNAs in human gastric cancer.
Materials and Methods: Published studies from PubMed online database were obtained using our search strategy. 138 studies were collected through search strategy and after removing duplicates, nonfulltext and nonrelated topics and abstracts, 21 studies were chosen. In 21 studies, a total of 180 miRNAs were observed which regulate stemness feature. For extracting miRNAs that regulate metastasis and drug resistance feature, we used CORMINE search engine and then the results were analyzed using R programming language that 162 miRNAs and 14 miRNAs were detected, respectively. Finally we found out that 6 miRNAs including miR-34a, miR-20a, miR-181a, miR-197, miR-21, miR-223 are common in these 3 features.
Results: Based on different sets of data, 6 miRNAs play a significant role in resistance to conventional therapy, stemness and metastasis in gastric cancer.
Conclusion: These data suggest that three mentioned properties in gastric cancer can regulated by common miRNAs, therefore, target these miRNAs or their targets suggested that they may be useful as biomarkers for gastric cancer.
Keywords: Gastric Cancer, Bioinformatics, miRNA, Metastasis, Stemness

Ps-12: Adult Muscle Derived Stem cells (MDSCs) with Myostatin Null Backgrounds Are Amenable to Pluripotent Conversion without Using Reprogramming Factors

Shenoy PS[1, 2], Bose B[1, 2*]

1. Yenepoya Research Centre, Stem Cells and Regenerative Medicine Centre, Yenepoya University, Mangalore, India
2. School of Biological Sciences, Nanyang Technological University, Singapore, Singapore
Email: Bipasha.bose@gmail.com

Background: Pluripotent conversion of somatic cells or adult stem cells without using Yamanaka factors/small molecules has rarely been reported except for spermatogonial stem cells or primordial germ cell that harbour some endogenous expression levels of pluripotency factors. Muscle derived stem cells (MDSCs) are multipotent adult stem cells located upstream of bonafide muscle stem cells/ satellite cells. Pluripotent conversion of MDSCs has never been reported. Myostatin (Mstn) / GDF-8, a myokine of TGFβ family, suppresses muscle growth. Studies related to myogenesis are carried out using myostatin null mice. Interestingly, we observed the expression of markers such as Leukemia Inhibitory Factor (LIF) and Leukemia inhibitory factor receptor (LIFR), and KlF4 in microarray analysis of MDSCs isolated from Mstn null mice, in contrast to WT-MDSCs. As LIF and LIF receptors and KLF4 are involved in mouse pluripotent stem cells, we explored the behaviour of Mstn null MDSCs when cultured in mouse pluripotent stem cell media containing LIF.
Materials and Methods: We isolated MDSCs using the well established preplate technique from the hind limb muscles of WT and Mstn null mice. Clones of WT and Mstn null MDSCs obtained using limiting dilution technique from both the genotypes were cultured in mouse ESC media containing LIF. We extensively characterized the ES-like colonies derived from the Mstn null MDSCs using qRT-PCR gene expression, analytical flow cytometry, immunofluorescence, Embryoid body formation and teratoma formation in immunocompromised Rag2- γ chain null mice. The cells (induced pluripotent stem cells, nonpluripotent parent and standard mouse ESC line) were subjected to Epitect DNA methylation arrays for 21 TGFβ family signature genes.
Results: Mstn null MDSCs exhibited ES-like colony formation from 45 days of culturing in mESC media, whereas the WT-MDSCs failed to form any such colonies. We obtained established colonies from Mstn null MDSCs at day 90 that were then sorted for SSEA-1, cultured and passaged. We termed these ES-like cells derived from Mstn null MDSCs as culture induced pluripotent stem cells (CiPSC). CiPSC expressed all the core pluripotency marker genes such as Oct4, Nanog, Sox-2, KLF4, c-Myc; formed shapely embryoid bodies and underwent trilineage differentiations *in vitro*. CiPSCs were >95% positive for Oct4 and SSEA-1 by flow cytometry. CiPSCs also exhibited normal karyotype through several passages (~30) and successfully formed teratomas in immunocompromised mice. Since Myostatin/GDF8 is a TGFβ family member, we assessed the epigenetic changes in the TGFβ family signature genes and identified the hypermethylation/silencing of BMP-2 that was further validated using qRT-PCR and western blots. We also identified BMP-2 silencing in the control mESC, but not the parental Mstn null MDSCs or else the WT-MDSCs.
Conclusion: Hence, we conclude that adult stem cells from the nongermline origin having Mstn null conditions are amenable to pluripotent conversion in ESC culture conditions. Also, we propose that lack of myostatin epigenetically rewires these adult stem cells by silencing BMP-2 via canonical BMP signaling pathway.
Keywords: Induced Pluripotency, Myostatin, Muscle Derived Stem Cells, BMP Signaling, Smad

Ps-13: Effect of Conditioned Medium of Morphine Treated Mesenchymal Stem Cells on The Neutral Red Cytotoxicity Test of 4T1 Cells

Delirezh N, Zand S*, Abtahi Froushani Z

Department of Microbiology, Faculty of Veterinary, University of Urmia, Urmia, Iran
Email: n.delirezh@urmia.ac.ir

Background: Mesenchymal Stem Cells (MSCs) are an important component of the tumor microenvironment and recruited by cancer cells to similarly aid in tumor growth and progression. Morphine is an opioid analgesic drug commonly used for pain relief in cancer patients. An increasing amount of evidences indicate a possibility that morphine causes immunosuppression on the hosts. This survey was set out to determine the influence of morphine on the interaction of MSCs on the 4T1

mammary carcinoma cell growth.
Materials and Methods: MSC was isolated by flashing the Tibia and femur bones of mice. After 14 days, MSCs were incubated for 24 h with 0, 1, 5 and 10 μM of Morphine. Then cells were cultured without serum for 24h and the conditioned medium (CM) was isolated. 4T1 cells were added to a 96 well plate at a density of 10000 cells per well, incubated for 24 h in a medium with isolated CM (50%) and FBS to allow attachment. After 72 h, viability of cells was evaluated with Neutral Red (NR) assay. Results: NR test indicated that the CM of MSCs treated with morphine could significantly increases proliferation of breast cancer cells (BCCs), in a dose dependent manner. Interestingly viability of cells cultured with CM of morphine treated MSCs is higher than viability of those who were cultured with CM of nonmorphine treated MSCs.
Conclusion: This data clearly showed that morphine affect MSCs to secret factors needed for BCCs survival.
Keywords: Mesenchymal Stem Cells, Conditioned Medium, Morphine, Breast Cancer Cells, Neutral Red

Ps-14: Neurobasal Medium/B27/N2 Supplements Influence Neurogenesis not Gliogenesis during Differentiation of Mouse Embryonic Stem Cells by Retinoic Acid

Ebadi R[1*], Kordi Tammandani DM[1], Ghaedi K[2, 3], Nasr Esfahani MH[3]

1. Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.
3. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: ebadi_reihane@yahoo.com

Background: The *in vitro* differentiation of embryonic stem (ES) cells to neurons is a promising approach to generate suitable cells for cell therapy of the nervous system. Obtaining high and reproducible percentages of neural cells is difficult. available methods attempt to promote ES cell differentiation towards neurons. These attempts involve the use of specific medium or addition of growth factors and growth supplements to the medium. Neurobasal medium is a basal medium when supplemented with B27 or N2 Supplement, contain antioxidants to reduce reactive oxygen damage, supports the growth of neuronal cells and maintenance the normal phenotype of these cells. In order to clarify the effect of neurobasal medium/B27/N2 supplements on neurogenesis and gliogenesis of mESC by retinoic acid, this study was performed. Retinoic acid (RA) is one of the main morphogenic factors which is used for neural differentiation of ES cells. RA induced neurosphere populations are a mix of neurons, astroctyes and oligodendrocytes.
Materials and Methods: We used Royan B20 cells as mESC. For neural induction, Cells cultured in hanging drops for embryoid body (EB) formation and then in suspension medium in presence of RA. Subsequently, EBs was seeded on plates for an additional 8 days in two different mediums. One, KO-DMEM supplemented with 5% ES-FCS and the other, Neurobasal medium supplemented with 5% ES-FCS, 2% B27 and 1% N2. The relative expression of neuronal markers (Map2 and Tuj1), glial markers such as asterocyte marker (Gfap) and oligodendrocyte marker (Olig2) were assessed on day 14 from the beginning of the experiment.
Results: Real time PCR data indicated that the expression levels of mature neuronal markers (Map2 and Tuj1) in neurons cultured in Neurobasal medium supplemented with ES-FCS and B27/N2 were higher than neurons cultured in KO-DMEM supplemented with ES-FCS while no significant difference was detected for astrocyte marker (Gfap) and oligodendrocyte marker (Olig2) between two groups.
Conclusion: We concluded that Neurobasal medium supplemented with B27/N2 affects the neurogenesis of mESCs but does not influence the gliogenesois as indicated by Gfap and Olig2 expression levels.
Keywords: Mouse Embryonic Stem Cells, Medium, Supplement, Neurogenesis, Gliogenesis

Ps-15: The Effect of Parvovirus B19 on Osteoblast Differentiation of Bone Marrow Mesenchymal Stem Cells

Elmi A[1*], Atashi A[2], Azad M[3], Gheibi N[1], Amiri Sh[1], Ajami M[2], Taghavi Z[2], Rasaei N[2], Mozaffar M[2]

1. Department of Medical Biotechnology, Qazvin University of Medical Sciences, Qazvin, Iran
2. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Medical Laboratory Science, Allied Medicine, Qazin University of Medical Science, Qazvin, Iran
Email: atashia@gmail.com

Background: One of the human bone marrow mesenchymal stem cells (hBM-MSCs) properties is the differentiation capability into osteocytes lineage. The differentiation should always be balanced, and the increase or decrease of this process can cause various diseases. On the other hand, osteoblastic cells regulate the hematopoietic stem cell niche. Parvovirus B19 (PVB19) is a pathogen which infects many types of cells and has remarkable tropism to bone marrow erythroid progenitor cells but it has been shown that the virus can enter and remain latent in hBM-MSCs as well.
Materials and Methods: hBM-MSCs were *in vitro* cultured under standard condition. Nucleofection was carried out to transfer a plasmid containing B19 genome into hBM-MSC. The cells were then differentiated into osteoblast using the osteoblast differentiation medium. Total RNA was extracted at 7th day posttransfection, and gene expression levels of RUNX2 and osteocalcin genes were examined by qRT-PCR.
Results: Data analysis from qRT-PCR in transfected cells showed a significant ($P<0.05$) increase in gene expression of studied genes.
Conclusion: Our findings for the first time suggest that PVB19 infection could increase osteoblastic differentiation of hBM-MSC. Future studies should focus on molecular mechanism of PVB19 – induced differentiation.
Keywords: Bone Marrow, Osteoblast Differentiation, Mesenchymal Stem Cell, Parvovirus B19

Ps-16: Metformin Treatment Causes Major Improvements in Electrophysiological Functions and Properties of Mouse Model of Multiple Sclerosis

Esmaeilnejad S*, Semnanian S, Javan M

Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mjavan@modares.ac.ir

Background: Multiple sclerosis (MS) is the most frequent neurological disease that causes disability in the young adults. It is hypothesized; immune system of MS patients wrongly launches attacks on its own oligodendrosrocytes and protective sheath surrounding nerve fibers of the central nervous system. Visual impairment is the most common complication of this disease. Metformin is one of the most widely used drugs for the treatment of type 2 diabetes mellitus. Previous studies have shown that metformin has effects on increasing proliferation, migration and differentiation of neural progenitor cells and strengthens neurogenesis. Thus, metformin could be considered as suitable candidate for the treatment of MS by increasing the regenerative capacity of endogenous neural stem and progenitor cells. Our aim is investigating the effect of metformin on regenerative capacity of endogenous stem cells in lysolecithin-induced mouse model of demyelination. In this study we examined the function of visual pathways by Visual Evoked Potential (VEP) recording as an indicator of visual pathway function and integrity.
Materials and Methods: To achieve mouse model of demyelination, Lysophosphatidylcholine (LPC) was injected intracerebrally into optic chiasm of male C57bl/6 mice brains. Metformin (200 mg/kg/day) was injected intraperitoneally once a day for 7 and 14 days. VEP recordings were performed in days 3, 7 and 14 after LPC injection. The data collected from these tests were compared with the baseline and also between different groups of our study.
Results: Metformin treatment decreases the adverse effects of LPC on electrophysiological functions and properties of lysolecithin induced mouse model of demyelination. The VEP data shows that conduction velocity is improved by metformin administration. These findings show that metformin treatment can maintain and improve the optic pathway function and integrity.
Conclusion: Treatment with metformin can maintain and improve visual impairments caused by LPC injection into optic chiasm of mouse model of demyelination. Presumably it is the result of protective effect of metformin on myelin destruction caused by LPC and likely metformin has regenerative effects by induction of proliferation of endogenous stem/progenitor cells. These stem/progenitor cells provide a vast reservoir for replacement of damaged cells. Our findings can introduce a new candidate for MS treatment. Nevertheless we need additional research to raise this claim quite confidently.
Keywords: Stem Cell, Multiple Sclerosis, Visual Evoked Potential, Metformin

Ps-17: Multiple Sclerosis and Stem Cells (Applications and Opportunities in Drug Discovery)

Esmaeilnejad S*, Semnanian S, Javan M

Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mjavan@modares.ac.ir

Background: Multiple sclerosis (MS) is the most common neurological disease affecting young adults and is the result of damage to myelin, the protective sheath surrounding nerve fibres of the central nervous system, the brain, spinal cord and optic nerve. In MS, the immune system wrongly attacks the myelin sheath covering nerve fibres in the brain and spinal cord, which can lead to symptoms such as vision loss, pain, fatigue and paralysis. MS causes progressive neurological disabilities. Yet, there is not any available treatment that can exert clinically meaningful impact on the symptoms of disease. Cell Therapy is an emerging form of treatment for neurodegenerative disorders such as MS. There are different strategies in stem cell therapy for MS such as using autologous hematopoietic stem cells to restore the individual's dysfunctional immune system and stop inflammation, utilizing the capacity of autologous mesenchymal or other cell populations for tissue repair and/or disease modification and cell replacement approaches to generate oligodendrocytes and induce demyelination by these cells. There are different sources of stem cells, including embryonic stem cells, adult stem cells and newly developed Induced Pluripotent Stem Cells (iPSCs). iPSCs, that can be derived from different sources, are a valuable tool for cell therapy purposes and/or developing research models for MS. iPSCs can be obtained by reprogramming somatic cells *in vitro* and *in vivo*. Application of stem cells for MS treatment gives us an important understanding of how myelin repair can be promoted, which could open up new practical and therapeutic areas for treatment development. In addition to the above mentioned application of stem cells, these cells provide a valuable advantage to study MS and testing newly developed drugs. iPSCs offer a limitless source of patient specific cells for researchers to understand disease mechanisms, recognize molecular targets and develop new drugs for this disease.
Materials and Methods: We have searched the PubMed databases comprehensively and accurately to find peer reviewed articles regarding stem cells and their applications in MS. We studied them carefully and selected the most prestigious and the most recent of them to ensure that all knowledge on this topic is discussed. Also we put meeting abstracts under precise consideration to ensure that all references have been investigated.
Results: In the reviewed the authors discussed the capabilities of iPSCs in multiple sclerosis (MS) treatment and research. In this review we emphasized on the great opportunity of using stem cells and also a number of challenges ahead and pointed to novel therapeutic strategies that can be applied for treatments for MS patients. At the end we discussed the opportunities provided by stem cells in drug discovery and development.
Conclusion: Application of stem cells in the treatment and drug discovery related to neurodegenerative diseases such as MS is a new emerging approach that their therapeutic potential is not far from mind.
Keywords: Multiple Sclerosis, Stem Cell, Reprogramming, Induced Pluripotent Stem Cells

Ps-18: Drug Repositioning as A New Approach in Neurodegenerative Diseases Treatment Using Stem/Progenitor Cells (with Emphasis on Multiple Sclerosis)

Esmaeilnejad S*, Semnanian S, Javan M

Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mjavan@modares.ac.ir

Background: Drug Repositioning or repurposing (DR) is a process of finding a new therapeutic use for existing drugs or drug candidates. This method has received much coverage in the scientific literature in recent years. DR is an attractive and timesaving strategy in drug development. Among the benefits of this approach are reduced time and costs, predetermined safety and pharmacokinetic properties of these drugs. In recent years there has been a significant movement toward the

use of computational methods for making initial repositioning suggestions. Another method for DR is "*in vitro* screening" of established drugs or molecules, to find new uses for them and finally introducing best candidates for DR. The most advanced methods for DR are High Throughput Screening (HTS) and High Content Screening (HCS) that allow scientists to test a large number of drugs in a short time. Using robotics, data processing/control software, liquid handling devices, and sensitive detectors, HTS and HCS allow a researcher to quickly conduct millions of chemical, genetic, or pharmacological tests. The results of these experiments provide starting points for DR. In stem cell research/therapy, the HCS enables the researcher to identify substances such as small molecules, peptides, siRNA and etc. that alter the phenotype of a cell in a desired manner. Many studies in DR for stem/progenitor cell production are ongoing and many studies have already been done in recent years. These data have provided a massive source of information about effects of known drugs for stem/progenitor cell production. In this study we have reviewed the latest articles in this area to provide a reliable source for the scientists interested to work in this exciting research area.
Materials and Methods: We have searched the PubMed databases comprehensively and accurately to find peer reviewed articles regarding DR in stem/progenitor research. We studied them carefully and selected the most prestigious and the most recent of them to ensure that all knowledge on this topic is discussed. Also we put meeting abstracts under precise consideration to ensure that all references have been investigated.
Results: In this review the authors discussed the potentials of using DR approach. The reviewed papers provided enormous data using HTS and HCS methods to find new uses for old drugs in stem cell research/therapy. We also emphasized on the great opportunity of using DR to develop new drugs for neurodegenerative diseases such as multiple sclerosis (MS).
Conclusion: The new approach of drug discovery that has been called DR is a new emerging approach in stem/progenitor cell research/therapy. The therapeutic potential of DR is very quickly accessible in terms of time and costs. We also highly recommend further investigations in this emerging research area.
Keywords: Stem Cell, Neurodegenerative Diseases, Drug Repositioning, Progenitor Cell, Multiple Sclerosis

Ps-19: Identification of Regulatory MiRNAs Involved in Cell Death during Neuronal Reprogramming

Falsafi N*, Fallahi H, Azadbakht M

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: Micro RNAs are small noncoding RNAs that are involved in post transcriptional regulation of genes and usually negatively regulate gene expression. MiRNA have shown to perform important roles during neurogen-esis and reprogramming different types of cells including PC12 cells into neurons. For example, a combination of transcription factors, miR-9 and miR-124 directly converted fibroblast to neuron-like cells. However, one of the challenges during conducting such reprogramming protocols is high rate of apoptosis that results in lower efficiency than expected. Despite many studies into this issue the major concern is still remained regarding unknown factors involved in apoptosis upon conversion. We aimed to identify important microRNAs that are involved in cell death during induction of PC12 by staurosporine as a model cell line for neurogenesis.
Materials and Methods: This work is divided in three different sections; In the first step, we have isolated RNAs from model cell line (PC12 cells treated with stauros porine in the culture medium). Next, RNA-sequencing was conducted. At the last step in depth bioinformatics analysis were performed to dissect the whole pathway of apoptosis with a focus on the regulatory miRNAs. Briefly, following identification of differentially expressed genes, miRNAs targeting these genes were analyzed. Network analysis and Gene Ontology studies were able to uncover many aspects of apoptosis in this cell line upon conversion into neuron.
Results: The results suggested involvement of many known miRNA is the apoptosis process. Besides, some new miRNAs were also identified that need further investigation to confirm their regulatory roles. It would be interesting to compare these results with those obtained from other cell lines to find common global pathways for apoptosis during direct somatic neurogenesis.
Conclusion: Finding regulatory elements including miRNA could be helpful in designing new conversion protocols with high efficiency rate. It could be done by including some small molecules to prevent induction of apoptosis.
Keywords: Apoptosis, Network Analysis, microRNAs, PC12 Cell Line

Ps-20: Identification of Transcription Factors Involved in Staurosporine Induced Cell Death in PC12 Cell Line

Falsafi N*, Fallahi H, Azadbakht M

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: Many neurodegenerative diseases are direct results of neuronal cell death. On the other hand, neuron cells lost their capacity for cell division. Therefore, we need to use alternative cell lines to produce neuron-like cells for regenerative studies. To do so, many model systems have been developed. One of the wellknown cell lines is PC12, which has been used for such studies due to its ease of culture, proliferation capacity. This cell line has been differentiated into neuronal cells using small molecules including NGF, PACAP and staurosporine. Among these small molecules, staurosporine is the wellknown for its role as inhibitor of protein kinase C (PKC) and is classified as an inhibitor of many serinethreonine and tyrosine kinases. Although many aspects of neurogenesis induction by this molecule has been documented at cellular and physiological level, the exact molecular impacts of this molecule on PC12 cell line upon neurogenesis has not been well studied. It is known that high dosages of this molecule would induce cell death during differentiation. Therefore, we have focused more on the impacts of staurosporine during neurogenesis using high through put data analysis.
Materials and Methods: PC12 cell line was maintained in RPMI 1640 supplemented with FBS (Fetal bovine serum). Then staurosporine were added to the medium. Next total RNA samples were extracted from treated and untreated culture using Qiagen RNAeasy minikit. RNA sequencing resulted in high quality pair ends reads. Follow up data analysis was conducted and transcription factors were identified using relevant web tools. Network analysis and gene ontology employed to unravel

the impacts of staurosporine in PC12.
Results: Differential gene expression analysis identified thousands of affected genes whose expression levels were affected by staurosporine. Network analysis for the apoptotic pathway identified the most affected transcription factors in PC12 during induction by this molecule.
Conclusion: The results of current study would be helpful in preventing cell death induction by small molecules during direct conversion of somatic cells into neurons. Besides, identifying these TFs would have usages in diagnosis, where neuronal loss would be linked to affected signaling pathways.
Keywords: RNA Sequencing, Cell Death, Network Analysis, Staurosporine, PC12

Ps-21: Transcriptome Comparison between Cardiac Cells Reprogramming in Mouse and Human

Falsafi N*, Soleimani T, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: One of the cause of mortality all over the world is cardiovascular disease. Many of these cases could be avoided by cell therapy, where damaged cardiac cells could be replaced by healthy one. However, isolation and *in vitro* generation of such cells is difficult. New strategies have been developed to overcome the shortages of these cell types including direct conversion or reprogramming of the somatic cells from patient into cardiac cells. In recent years, many researchers have tested several regulatory elements including transcription factors, chemical cocktails or combination of transcription factors and microRNAs to convert different fibroblast cells such as foreskin fibroblast, lung and cardiac fibroblasts into cardiac-like cells. However, their effort lag behind due to low conversion efficiency. The big issue in this regard is the lack of sufficient knowledge of molecular route involved in this conversion. With this in mind, in this way, we have studied five datasets from mouse and human during conversion of fibroblasts into cardiac to identify the most important regulatory elements involved.
Materials and Methods: First, we have isolated differentially expressed genes with fold change greater or less than 2. Then transcription factors of differentially expressed genes which expressed differently were separated. We constructed two networks for human and mouse studies with DE-TFs and their target genes. Finally the main DE-TFs with highest MCODE score were reported. Gene ontology analysis was used to uncover affected pathways.
Results: Network analysis base on MCODE score have shown that RUNX2, MYB and TCF4 are the most important TFs during direct conversion of human fibroblasts. While, RUNX2, EBF1, FLI1, BRCA1, RCOR3 and IRF8 are similar TFs among mouse data sets and had the highest scores.
Conclusion: These findings would present new possibility and novel targets for the conversion strategies. By modulating the functional level of these TFs, it would be possible to directly convert fibroblasts to cardiac cells with higher efficiency and yield.
Keywords: Transcriptome Analysis, Cardiogenesis, Reprogramming, Somatic Cells

Ps-22: Differentiation Capacity of iPSCs Derived from Different Somatic Cells in Blood Cells Generation

Farhangian M*, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: fallahi.raziuni@gmail.com

Background: Hematopoiesis is a process during which blood cells are produced. Establishment and maintenance of the blood system relies on selfrenewing hematopoietic stem cells (HSCs) that normally reside in small numbers in the bone marrow niche of adult mammals. Blood cells can be also generated *in vitro* from induced pluripotent stem cells (iPSCs) through somatic cell reprogramming or by direct conversion. Direct reprogramming of somatic cells into iPSCs provides an opportunity to develop novel personalized treatment options for numerous diseases and to advance current approaches for cell-based drug discoveries and disease modeling. There are subtle differences in the differential capacity of iPSCs lines in this regard. Some iPSCs lines has been derived from blood and fibroblast somatic cells are suitable for blood cells differentiation. It is a significance concern for their use in clinical application. To identify factors that are involved in the differentiation capacity of iPSCs, we compared gene expressions between different iPSCs lines in the undifferentiated state, then key TFs and micorRNAs determinant of such differences were analyzed.
Materials and Methods: In this study for getting differentiation capacity in the iPSCs, an insilico approach was employed. First we obtained the differential gene expression list for each study. Then gene ontology analyses were conducted to find the most affected pathways. Most important TFs and miRNAs were studied and using all these knowledge a core regulatory network was constructed for comparison.
Results: Our approach revealed many aspects of differential determinant in stem cells. We could correlate some of these differences to key regulatory elements both miRNAs and TFs. Network analysis uncovered major differences in core regulatory network between these cells.
Conclusion: These analyses would be useful for clinical application specially cell therapy applications. We have identified the source of differences in the hematopoietic differentiation from iPSCs. We hope that our analysis facilitates selection of iPSC lines for hematopoietic differentiation by optimizing the current protocols.
Keywords: iPSCs, ESCs, Hematopoiesis, Network Analysis, miRNAs

Ps-23: The Regulatory Aspects of Transcriptome between Different iPSCs Resulting from Different Somatic Cells

Farhangian M*, Azarafrouz F, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: fallahi.raziuni@gmail.com

Background: The genes involved in cell cycle are of most important genes undergo an expression pattern shift during reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). The capacity of stem cells to differentiate is crucial for stem cell therapy and pathogenic studies. However, conversion of somatics cells into stem cells depend on the source cells genomics. In this regards, different cells show variation in their conversion into iP-SCs. Besides, the resulting iPSCs

show different degree of similarities to ESCs as a standard for cell therapy applications. Regulatory elements play a key role in determining the primary capacity of the somatic cells to be converted to iPSCs. However, such regulatory differences between the original spmatic cells that determine the final efficiency rates of conversion have not been studied. To find these elements including miRNAs and transcription factors, we have conducted an in slico analyses using microarray data obtained from different somatic cells and their iPSCs counterparts.
Materials and Methods: To keep consistence regarding the conversion protocol and media compositions microarray data first selected based on a single platform microarray. Following extracting differentially expressed genes, the list subjected to regulatory elemnt analysis. Then an integrated network was constructed using miRNAs and TFS involved in the regulation of DE gene list.
Results: Our results identified several miRNAs and TFs for which different expression pattern were observed in different cells. The network revealed the most significant differences among different resultant iPSCs. Gene ontology analyses uncovered the molecular and functional pathways affected in different iPSCs.
Conclusion: Our findings would be useful for finding a common protocol to efficiently produce more homogenous iPSCs for use in cell therapy. For example, the function of many of these miRNAs and TFs could be attenuated using small molecules that could be integrated into the conversion protocols.
Keywords: iPSCs, miRNA, Transcription Factors, Microarray Analysis, Direct Conversion

Ps-24: Cytokines TNF-α, IL-6, IL-17F, and IL-4 Differentially Affect Osteogenic Differentiation of Human Adipose Stem Cells

Forouzanfar T[1], Bastidas Coral AP[2], Bakker AD[2], Zandieh-Doulabi B[2], Kleverlaan CJ[3], Bravenboer N[4], Klein Nulend J[4*]

1. Department of Oral and Maxillofacial Surgery, VU University Medical Center, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands
2. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije University, Amsterdam, Netherlands
3. Dental Materials Science, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije University, Amsterdam, Netherlands
4. Department of Clinical Chemistry, VU University Medical Center, Amsterdam, Netherlands
Email: j.kleinnulend@acta.nl

Background: During the initial stages of bone repair, pro-inflammatory cytokines are released within the injury site, quickly followed by a shift to anti-inflammatory cytokines. The effect of pro and anti-inflammatory cytokines on osteogenic differentiation of mesenchymal stem cells is controversial. Here, we investigated the effect of the proinflammatory cytokines TNF-α, IL-6, IL-8, and IL-17F, and the anti-inflammatory cytokine IL-4 on proliferation and osteogenic differentiation of human adipose stem cells (hASCs).
Materials and Methods: hASCs were treated with TNF-α, IL-6, IL-8, IL-17F, or IL-4 (10 ng/ml) for 72 h mimicking bone repair.
Results: TNF-α reduced collagen type I gene expression, but increased hASC proliferation and ALP activity. IL-6 also strongly enhanced ALP activity (18-fold), as well as bone nodule formation by hASCs. IL-8 did not affect proliferation or osteogenic gene expression, but reduced bone nodule formation. IL-17F decreased hASC proliferation, but enhanced ALP activity. IL-4 enhanced osteocalcin gene expression and ALP activity, but reduced RUNX2 gene expression and bone nodule formation.
Conclusion: In conclusion, all cytokines studied have both enhancing and reducing effects on osteogenic differentiation of hASCs, even when applied for 72 h only. Some cytokines, specifically IL-6, may be suitable to induce osteogenic differentiation of mesenchymal stem cells as a strategy for enhancing bone repair.
Keyword: Adipose Stem Cells, Mesenchymal Stem Cells, Osteogenic Differentiation, Cytokines, Bone Repair

Ps-25: Analysis of Fibronectin Type III Domain Containing 5 (FNDC5) Expression in Breast Cancer Cell Lines

Ghazvini Zadegan F*, Ghaedi K, Nasr Esfahani MH

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: f.ghazvini75@gmail.com

Background: The Peroxisome proliferator activated receptor (PPAR) γ coactivator 1α (PGC1α) transcriptional coactivator is involved in many biological programs related to energy metabolism. The expression of PGC1α induced fibronectin type III domain containing 5 (FNDC5) expression in white adipose tissue (WAT). FNDC5/Irisin, a novel hormone in the regulation of energy balance, is involved with carbohydrate and lipid metabolism. Irisin is increased by exercise responsive PGC-1α to activate thermogenic programs in white adipose tissue suggesting that it has role in metabolic homeostasis. Energy imbalance is correlated with increased morbidity and mortality from all causes of cancer, including cancer of the breast, colon and prostate. Significant positive association between energy intake and breast cancer risk were identified in different studies, and more recent evidence showed that an increased risk of breast cancer was related with relatively high energy consumption among humans. In addition, a majority of the cohort studies of calorie restriction in human support a potential beneficial influence on breast cancer risk.
Materials and Methods: Therefore, FNDC5 expression levels were detected in three breast cancer cell lines i.e. MCF7, SKBR3, and MCF10A by real-time PCR. Results: Data revealed a significant elevation in expression level of FNDC5 in SKBR3 cell line compare to MCF7 and control cell lines.
Conclusion: A positive association with FNDC5 expression and metastatic cell line (SKBR3) suggested that the potential application of FNDC5 as a facilitator of invasion and metastasis of adenocarcinoma cells.
Keywords: FNDC5, Breast Cancer Cell Lines, Expression Level

Ps-26: Effect of Culture Medium on *Ex Vivo* Expansion of Peripheral Blood Derived Natural Killer Cells

Ghodsi A[1, 2*], Shekari F[1], Ebrahimi M[1], Hassani SN[1]

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

2. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: nafiseh.hassani@gmail.com

Background: Immunotherapy is relatively a new and alternative way for cancer therapy and it reawakens the potential of immune system to fight cancer. Interest has grown toward the clinical application of NK (natural killer) cells which are lymphocytes of the innate immune system. Given that these cells only compose 5 to 15 percent of human peripheral blood mononuclear cells; the number of NK cells in blood is highly variable between individuals and still not enough for injection to the patient; expansion of NK cells is considered an essential step.
Materials and Methods: In this study, two media, RPMI 1640 and SCGM, were compared for their effect on NK cell expansion. Peripheral blood mononuclear cells containing NK cells were obtained from healthy individuals and expanded *in vitro* with inductions of K562 cell line and 500 IU IL-2 for 1-2 weeks in T25 flasks and the fold expansion was reported at the end of the culture.
Results: NK cell loss was observed using RPMI after 7 days of culture. However, starting with 4x105 NK cells in SCGM medium resulted in more than 50 fold expansion with more than 90% viability after two weeks.
Conclusion: We have successfully set up an *ex vivo* NK cell expansion protocol using SCGM medium. This laboratoryscale expansion has been established to provide a platform for NK cell application in clinical settings.
Keywords: Natural Killer Cells, Immunotherapy, Expansion

Ps-27: The Influence of Cerebrospinal Fluid Accompanied by Schwann Extracted Medium on Differentiation of Bone Marrow Mesenchymal Stem Cells into Neuron-like Cells *In Vitro*

Goudarzi G*, Ghasemi Hmidabadi H, Esmaeilnejad Moghaddam A

Department of Anatomy and Cell Biology, School of Medicine, Mazandaran University of Medical sciences, Sari, Iran
Email: mog1339@gmail.com

Background: Cerebrospinal fluid (CSF) has a broad range of molecules and neurotrophic factors that essential for neurogenesis, Schwann cells (SCs) are special neurogliocytes that have a strong ability to induce nerve regeneration due to release of variety of neurotrophic factors. Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells that can differentiate into neural-like cells under the induction of appropriate growth factors. This study aims to differentiate BMSCs into Neuron-like Cells using CSF, Schwann Extracted Medium (SCM), and the combination of CSF and SCM.
Materials and Methods: Rat BMSCs were isolated and characterized. The CSF was prepared from the Cisterna magna of 19-day-old Wistar rat embryos. The SCs from adult rat Sciatic nerve were isolated and cultured. Then the SCM were collected every 3 day. The BMSCs were induced by either 5% CSF (CSF group), 50% (SCM group), or CSF plus SCM (CSM group) for 12 days. Morphology of differentiated cells was examined by inverted microscope and axonal outgrowth was measured using Image J software. In addition, the expression of neural specific markers (Nestin and MAP-2) was examined by immunocytochemistry. Nissl bodies were stained in differentiated cells by Cresyl violet. The expression of neural genes (Nestin and β-III tubulin) was examined in comparison to control group by PCR assay. The data were analyzed with t student test.
Results: We observed specific neuronal morphology in the differentiated cells. The maximum axon length was seen in the CSM group on the 12th day of induction. Immunocytochemistry results showed that the neural progenitor marker (Nestin) was expressed in all treated groups. However, MAP-2, as a mature neural marker, was only expressed in the CSM group. Nissl bodies were detected as darkblue particles in the cytoplasm of treated cells. The PCR analysis showed expression of Nestin and β-III tubulin in treated group.
Conclusion: The findings suggest that combination of CSF and SCM could provide neurotrophic factors for differentiation neuron-like cells from BMSCs *In vitro* in a similar manner to *in vivo*.
Keywords: BMSCs, Cerebrospinal Fluid, Schwann Extracted Medium, Neuron-Like Cell, Differentiation

Ps-29: SDF -1α /CXCR4 Pathway in Hair Follicle Stem Cell Transplanted Rats Accelerated Cutaneous Wound Healing

Heidari F[1*], Yari A[2], Nobakht M[3], Heidari M[4]

1. Medical Record Unit, Nekoei Hedayati Hospital, Qom, Iran
2. Department of Anatomy, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran
3. Department of Anatomy, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran
4. Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: heidari_anatomy@yahoo.com

Background: The SDF-1/CXCR4 axis is important in the recruitment of stem cells to the sites of injury. Some organs respond to damage by increasing expression of SDF-1/CXCR4. However, the possible role of this axis in skin wound healing specially in the case of Hair Follicle Stem Cell (HFSCs) applied wounds has received low attention to date.
Materials and Methods: Animals (male rats) were divided into three groups: 1. Control (nontreated), 2. Vehicle (PBS) and 3. HFSCs (treated with Hair Follicle Stem Cells). The Bulge region of rat whiskers was isolated and cultured in DMEM/F12, then transplanted to wound site. At the end of the treatment period, in three different days (3, 7 and 14), Morphological and histological assessments, and molecular assays (ELISA and RT-PCR) for VEGF, SDF-1a and CXCR4 were performed.
Results: Morphological analysis of wounds exhibited early wound closure in HFSCs group. In histological analysis, the diameter of epidermis, Amount of collagen formation and wound healing percent in HFSCs group were significant compared with control group ($P<0.05$). in molecular assays, angiogenesis (VEGF level), SDF-1a and CXCR4 expression and protein secretion in HFSCs groups were more significant compare with control group ($P<0.05$).
Conclusion: Transplantation of HFSCs, induce secretion of SDF-1 and CXCR4 expression in wound bed which play important role in angiogenesis and accelerate cutaneous wound healing.
Keywords: HFSCs, Wound Healing, SDF-1a, CXCR4, Hair follicle

Ps-30: Evaluating Effect of Mesenchymal Stem Cells Supernatant in Ameliorating Experimental Autoimmune Encephalitis

Heidari Barchi Nezhad R[1*], Abtahi Froushani SM[1], Asadi F[2]

1. Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
2. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: meysamabtahi@hotmail.com

Background: Bone marrow derived cells culture supernatant have recently been described to give rise to multiple mesenchymal phenotypes. The aim of the current study was to evaluate the ability of bone marrow derived mesenchymal stem cells supernatant in ameliorating of experimental autoimmune encephalitis (EAE).
Materials and Methods: EAE was induced in Wister rats by guinea pig spinal cord homogenates and complete Freund's adjuvant. Therapies were initiated at day 12 post immunization when the mice developed a disability score with 2×10^6 of routine adherent mesenchymal stem cell or non adherent bone marrow cells. After day 33, the mice were sacrificed and the effects of cell therapy were investigated
Results: Clinical scores, leukocyte infiltration and lymphocyte proliferation were significantly diminished in a similar pattern in both treatment groups. Body weight increased significantly in the both treated groups compared to EAE rat without treatment
Conclusion: Mesenchymal stem cells supernatant may be a simple and costeffective way to cell therapy of autoimmune disorder such as multiple sclerosis
Keywords: Bone Marrow Cell, EAE, Multiple Sclerosis,

Ps-31: Optimization of Limbal Stem Cell Explant Culture and Identification Methods

Javid D[1*], Nosrati A[2], Zargari S[1], Hamidi D[1]

1. Department of Medical Biochemistry, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Medical Genetic, Mashhad University of Medical Sciences, Mashhad, Iran
Email: dhamidialam@yahoo.com

Background: Limbal epithelial stem cells (LSCs) encircle the cornea are responsible for maintaining the corneal transparency and integrity. Innate and acquired disorders defects on LSCs functionality and physiology can cause limbal stem cell deficiency (LSCD). Patients with LSCD lost their eyesight due to the invasion of conjunctival cells. Cultured limbal epithelial stem cell transplantation (CLET) seems to be the most applicable method for treatment of LSCD. In present study, the unique LSCs explant culture using fibrin glue(FG) in twothree dimensional (2D-3D) systems has been done. The immunohistochemistry(IHC) and immunofluorescent(IF) staining method was used for nucleoprotein P63 and transmembrane ATP-binding cast G2 (ABCG2) to confirm the development of LSCs on human amniotic membrane(HAM). In order to preserve natural morphology of LSCs and HAM, tissue fixation optimized. Moreover, the modified IF protocol applied for ABCG2.
Materials and Methods: The corneal rims received from Khatamal-Anbia hospital. Then, rims cultivated on the HAM. FG applied in 2D-3D explant systems for LSCs development. The explant set incubated for 14 days. To confirm the expanded LSCs on HAM, IHC and IF and modified IF for P63 and ABCG2 have been applied. HAM was fixed by four common fixatives and the blocking step in IF procedure optimized by comparison of different sera.
Results: Our investigations revealed that LSCs expanded on HAM. The results showed that the explant set using 3D-FG and amniotic membrane improves the quantities ($P<0.05$) of expanded LSCs. Optimized IF protocol showed that using sera has no effect on quality of immunostaining results. The histological investigations showed that methanol at -20°C for 10 minutes is the best fixative to preserve structure of LSCs on HAM.
Conclusion: The results showed that our explants set impressively improve LSCs expansion on HAM which is more efficient for CLET.
Keywords: Limbal Stem Cell, 3D Explant Culture, Immunostaining, Human Amniotic Membrane

Ps-32: Comparison of Metabolic Sates in Mouse Pluripotent Cells via Context Specific Metabolic Reconstruction

Khassafi F[1, 2*], Meymandi Parizi F[3], Sharif Zarchi A[1, 4], Karimi Jafari M[3], Totonchi M[1, 5]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
3. Department of Bioinformatics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
4. Department of Computer Engineering, Sharif University of Technology, Tehran, Iran.
5. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: m.totonchi@royaninstitute.org

Background: Different types of pluripotent stem cells are similar in their pluripotency potential but they show critical discrepancies in some other traits. These differences lead to define different states of pluripotency *in vivo* and *in vitro*. One of the challenging discriminations in various pluripotent cells is metabolic states. A metabolic network (MN) consists of all interconnected biochemical reactions occurring in a cell can be modeled insilico. MN modeling is one of the viable methods to study cell metabolism that connects genotype to the phenotype of a cell and describes its following physiological behavior in different conditions. Since eukaryotic cell types have more complex MNs and differ in their active metabolism, we need to analysis each cell type by contextspecific metabolic model reconstruction.
Materials and Methods: Here we reconstructed mouse pluripotent cell type specific MNs. We used transcriptomic data of ICM (Inner Cell Mass), ESC (Embryonic Stem Cell) and iPSC (induced Pluripotent Stem Cell) and also the genomescale mouse metabolic model known as iMM1415. We used the mCADRE algorithm which is one of the powerful algorithms for reconstruction of specific models. After its refinement by a predictive network model, we compared the pluripotent cell metabolic models as well as the mouse fibroblast model as a differentiated cell control.
Results: Pluripotent cell MNs demonstrate similarities in their reactions with each other and are far from fibroblast model. In fact, they have distinguished metabolic states from fibroblast cells. Besides, we focused on metabolic pathways were vastly distinct among the pluripotent states. Flux Variability Analysis (FVA) revealed a different number of reactions and vari-

ous fluxes in energy metabolism and nucleotide biosynthesis related pathways in each cell type. Reaction Flux in glycolysis and pyruvate metabolism were highly different in iPSCs versus Fibroblast model. Essential gene analysis also represents different metabolic genes that affect cell growth in each pluripotent models.
Conclusion: Pathway analysis of specific MNs pluripotent cells shed light on metabolic regulations of pluripotency. The results assist us in a more efficient generation and maintenance of pluripotent cells *in vitro* and investigating the mechanisms of reprogramming and differentiation of these cells into other lineages using metabolic alterations.
Keywords: Metabolic Network, Pluripotent Stem Cell, Metabolism, Pathway Analysis

Ps-33: Apoptosis Induction in A Glioblastoma Multiforme Cell Line by Temozolomide and Tranilast

Khazaei M*, Pazhouhi M, Ansarian A
Fertility and Infertility Research Center, Kermanshah University of Medical Science, Kermanshah, Iran
Email: mkhazaei1345@yahoo.com

Background: Glioblastoma multiforme (GBM) is the most malignant primary brain tumor. Temozolomide (TMZ) is a chemotherapeutic agent that has been used in GBM treatment. Resistance to TMZ is a major obstacle to successful treatment of GBM. Tranilast is an antiallergic drug with antiproliferation property. The aim of the present study was to investigate the synergic effect of TMZ and tranilast on the human GBM cell line (U87MG).
Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS. Cells were treated with 20 μM TMZ, the maximum concentration of TMZ in the brains and/or 100 μM tranilast. Lactate dehydrogenase assay was used to determine TMZ and tranilast cytotoxicity. The effect of TMZ and tranilast on U87MG apoptosis was evaluated by TUNEL staining. Acridine orange and ethidium bromide staining was used for evaluation nuclear morphological change in cells. Data were analyzed by oneway ANOVA and $P<0.05$ was considered significant.
Results: TMZ and tranilast had a significant dose dependent cytotoxic effect on U87MG cells. Apoptosis was induced by TMZ and/or tranilast. Live cell with normal morphology are abundant in control group, early apoptosis occurred in the group treated with TMZ, both early and late apoptosis occurred in the group treated with tranilast, and in combination treatment group nearly all the cells are in late stage of apoptosis and few cell in early stage are present.
Conclusion: The combination of TMZ and tranilast for the treatment of human glioblastoma patient may result in a desirable clinical outcome.
Keywords: Drug Combination, Glioblastoma Multiforme, Temozolomide, Tranilast

Ps-34: Treatment of Alzheimer's Disease Using Stem Cells: A Concept Map

Khazaeli AR

Faculty of Pharmaceutical Sciences, Islamic Azad University, Tehran, Iran
Email: Themicrokh@gmail.com

Background: For a long time, the drugs prescribed for Alzheimer patients have had moderate effects, and only inhibit the neurodegeneration process, and come with a high number of adverse effects. This has led Researchers to look into the stem cell field in search of regenerating the lost and damaged tissues.
Materials and Methods: This Poster aims to utilize concept maps to better explain the novel approaches to restoring the effects of Alzheimer's using stem cells. Each fact is presented in a "Node" and the descriptive links between them can aid in comprehending a mass amount of data in minimal amount of time.
Results: As a Natural occurring with concept maps, potential novelties are formed in the process; giving the necessary scope on the path ahead.
Conclusion: Ultimately, the disease and its potential connection with stem cells is reviewed from different aspects. The aim of this category of posters is to help the readers quickly find the info they need by simply following the links that correspond to the next node.
Keywords: Concept Map, Alzheimer, Stem Cells, Ethics, Pluripotent

Ps-35: Immunomodulatory Effect of Oligodendrocyte Lineage-like Cells Transdifferentiate from Adipose Mesenchymal Stem Cells

Khoshnoodi M[1, 2*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Physiology, Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mjavan@modares.ac.ir

Background: Differentiation of stem cells into Oligodendrocyte Precursor Cells and transplanting them is a novel strategy for the repair of different demyelination diseases. Mesenchymal stem cells (MSC) have emerged as alternative sources of stem cells for regenerative medicine because of their multipotency and strong immuneregulatory properties. In this study, MSCs were isolated from human's adipose tissue and transfected with the Olig2 gene as a differentiation inducer which plays an important role in the oligodendrogenic pathway and Evaluating the immunomodulatory properties and immunogenicity of OPCs derived from AD-MSCs.
Materials and Methods: AD-MSCs were transfected with pCCL PGK EGFP Olig2 vector which constitutively expressed Olige2 gene. Thus, green cells were identified as manipulated cells. These cells were cultured in OPC induction medium for 21 days. Following induction, the expression of stagespecific markers was studied by Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR), immunocytochemistry and also mixed lymphocyte reaction was performed to evaluate their immunomodulatory properties and immunogenicity.
Results: Following the differentiation procedure, cell morphology changed from long, spindle like cells to small, round cell bodies with sprouted and also the RT-qPCR results demonstrated that in AD-MSCS-derived OPCs, the expression of Sox10, NG2, PDGFRα, Olig2 and Plp was upregulated during the course of differentiation. Furthermore, MLR results indicate that AD-MSCs derived OPC retained immunosuppressive capacity and low immunogenicity
Conclusion: Our findings demonstrated that the expression of Olig2 in AD-MSCS induces the development of oligodendro-

cyte progenitors as revealed by the emergence of oligodendrocyte markers. And also we identified both low immunogenicity and strong immunosuppressive function of AD-MSCs after OPC transdifferentiation. AD-MSCS could be programmed into oligodendrocyte progenitors and considered as a simple and valuable source for the cell therapy of neurodegenerative diseases.
Keywords: Mesenchymal Stem Cell, Oligodendrocyte Precursor Cell, Immunomodulatory, Immunogenicity, Transdifferentiation

Ps-36: Therapeutic Potential of Mesenchymal Stem Cell and its Conditioned Medium for Hepatocyte Regeneration

Khosravi Farsani S[1, 2*], Zaminy A[3], Khosravi Farsani L[4], Kazemi S[1], Hashemzadeh M[1]

1. Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

2. Department of Anatomical Sciences, Guilan University of Medical Sciences, Rasht, Iran

3. Faculty of Dentistry, Shahrekord University of Medical Sciences, Shahrekord, Iran

4. Department of Anatomical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran

Email: mchalesh@yahoo.com

Background: Liver ischemia reperfusion injury (IRI) is inevitable during surgical procedures including transplantation and resection and is characterized by hepatocellular injury. It has also been proposed as an underlying mechanism responsible for the dysfunction and injury of other remote organs as well. Hepatocytes are mostly affected cells of the liver cell types during I/R injury. Therapeutic strategies to reduce IRI and accelerate regeneration could offer major benefits. In the present study we evaluated the potential therapeutic roles of mesenchymal stem cells (MSC) and MSC-conditioned medium (MSC-CM) on hepatocytes after acute liver failure.
Materials and Methods: MSC were isolated from mouse bone marrow and checked for surface markers for characterization. CM was also made from MSC. Afterwards, they were transplanted into a model of IRI in NMRI mice. At 5h, 24h, 1w after reperfusion, serum biochemical parameters and genes expression were evaluated.
Results: We showed that transplanted MSC and CM could decrease markers of liver injury, such as alanineaminotransferase, aspartateaminotransferase. They had also the ability to improve gene experession in hepatocytes in mice with acute liver injury.
Conclusion: It was observed that CM protects liver against IRI and can replace progenitor cell transplantation.
Keywords: Mesenchymal Stem Cells, Acute Liver Failure, Ischemia, Reperfusion

Ps-37: DNA Methylation Dynamics during Myogenic Differentiation

Lafta H[*], Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran

Email: h.fallahi@razi.ac.ir

Background: Regulation of gene expression occurs at different level including transcription, translation and steps hereafter. Such regulatory machinery has been uncovered during cell differentiation and cell fate determination. However, another layer of regulation imposed by epigenetic modifications is shown to be more important in such events. Epigenetic changes including DNA methylation, histone methylation and chromosomal remodeling are main regulators of gene expression in mammals. Among these, DNA methylation plays critical role during development. As such upon differentiation an intensive DAN methylation pattern has been documented. Unfortunately, this event is poorly studied during myogenic differentiation, where muscle cells are formed from their progenitors.
Materials and Methods: To address this issue, we have conducted an insilico study using previously published data sets to unveil the pattern of DNA methylation during this process. Highthrough put data containing global DNA methylation pattern for the myoblasts differentiation steps were obtained from NCBI. Differentially methylated regions were mapped to the genome and proximal genes and regions were extracted. Besides, the list of differentially expressed genes at similar steps was obtained by analysing microarray data. Next, DNA methylation and gene expression patterns were integrated to understand the impacts of methylation dynamics on differential gene expression. Finally, gene regulatory networks were constructed and analyzed. Then, gene ontology of the affected pathways was assessed to better categorise the global dynamic changes in gene expression pattern.
Results: Our results indicated that global DNA methylation level increases as the differentiation of the myoblasts proceeded. Expectedly, these methylation patterns are entirely distinct from those of mesenchymal stem cells. Network and gene ontology analyses revealed substantial alteration in gene expression pattern as a result of differential DNA methylation upon differentiation.
Conclusion: These results would be beneficial to manipulate the methylation pattern using small molecules. If successful next step would be optimisation of differentiation protocols to produce high quality muscle cells from stem cells by including such small molecules.
Keywords: DNA Methylation, Network Analysis, Myogenic Differentiation, Microarray Data

Ps-38: Gene Regulatory Network of Stemness Marker SOX2 in Gastric Cancer Tumorigenesis

Lafta H[*], Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran

Email: h.fallahi@razi.ac.ir

Background: Due to poor diagnosis and lack of proper treatment, gastric cancer causes many deaths annually worldwide. Cancer stem cell believes to be involved in different aspects of this disease giving this tumor invasion capacity and resistance to many cancer therapy approaches. Similar to other types of cancer genetic alteration appears to play vital role in conversion of somatic cells to cancer stem cells in the gastric cancer. Previous studies have identified many such genetic markers including aberrant expression of SOX2, which is one of the stem cell markers. Although, the role of SOX2 and its regulatory network in stem cell biology is notably clear, the question remains if SOX2 would have similar role in gastric cancer stem cells. Despite many efforts, the exact role of this transcription factor in gastric cancer Tumorigenesis has not been discussed yet. This is partly due to the role of this transcription factor in regulation of global proliferation and maintenance of different types of cells.
Materials and Methods: There are some reports on blocking

SOX2 transcriptional activity in gastric cancer cell lines and assessing its absence impacts. In order to address this issue, we have analyzed microarray data obtained from such studies and constructed a gene regulatory network in presence and absences of SOX2. We have then assessed the impacts over time in a time course manner to understand versatile changes induced by the absence of this TF in the core regulatory network.
Results: Our gene ontology results indicated that similar to the role of SOX2 in stem cells proliferation, antiapoptotic activity of SOX2 is the major contributor toward uncontrolled cell growth in gastric cancer cells. Additionally, some of the key cell cycle related genes including cyclins were also affected by removing SOX2 transcriptional activity. Besides, absence of SOX2 activity has versatile impacts on other transcriptional activity and signalling pathways. The comparison between SOX2 regulatory network in embryonic stem cells and gastric cancer cells revealed significant similarities and differences between these two cell lines.
Conclusion: Altogether, core regulatory network analysis indicted involvement of SOX2 in key aspects of proliferation including cell division and apoptosis. These findings could present new opportunity for cancer therapy using SOX2 as main target.
Keywords: SOX2, Stemness Marker, Cell Cycle, Gene Regulatory Network, Cancer Stem Cell

Ps-39: To Evaluate The Effect of Intravitreal Mesenchymal Stem Cell Conditioned Medium for Treatment of Experimental Endotoxin Induced Uveitis in Rabbit Model

Mehrabian Sh[1], Mehrabian E[2]

1. School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Email: miremadodinmehrabian@gmail.com

Background: One of the most important limitations of stem cell therapy is their short life time. Previous investigations showed similar characteristics between stem cells and cancer cells. Stem cell conditioned medium may be an alternative therapy which could be safer than stem cell therapy. In previous studies antiinflammatory, immunomodulatory and neuroprotective effect of conditioned medium proven.
Materials and Methods: The rabbits divided into three groups: 1st include 5 rabbit and received condition medium lonely. 2nd group include 10 rabbit which received 0.1cc lipopolysaccharides and 0.1cc normal saline. 3rd group include 15 rabbit who received 0.1cc lipopolysaccharides and 0.1cc conditioned medium. We evaluate clinical sign of inflammation, anterior chamber and vitrous protein, anterior chamber and vitrous TNFα and finally the eyes enucleated and evaluated pathologically.
Results: Anterior chamber protein in case and control group was 777 pgr/ml and 1267 pgr/ml which reach significant value (P value: 0.01). Vitrous proteins were 477 and 944 pgr/ml in case and control groups which was significant (P value: 0.01). TNF concentration in anterior chamber were 11.3 and 11.01 in case and control group that did not reach significant value (P value: 0.85). TNF concentration in vitrous were 9.84 and 11.63 in case and control group which was not a significant difference (P value: 0.88). case and control groups had no significant difference when we consider clinical symptoms and pathological grading (both P value>0.05).
Conclusion: According to our results conditioned medium has positive effect on reduction of protein concentration in both anterior chamber and vitrous in endotoxin induced uveitis in rabbits.
Keywords: Mesenchymal Stem Cell Conditioned Medium, Uveitis, Animal Model, Endotoxin Induced Uveitis

Ps-40: How Cancer Stem Cell Theory Give Rise to Anaplastic and Papillary Thyroid Carcinomas in Human

Mehrabian E[1*], Mehrabian Sh[1], Fallahi J[2], Karimi A[2], Bahmanzadegan MH[1]

1. Department of Molecular Medicine, Stem Cell Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: miremadodinmehrabian@gmail.com

Background: Papillary thyroid carcinomas (PTCs) are the most common type of thyroid cancers and Anaplastic thyroid carcinomas (ATCs) remain of the most lethal human cancers. The cancer stem cell (CSC) hypothesis suggests a small population of stem-like cells that generate and sustain tumor cells, and are characterized by their ability for selfrenewal, proliferation, tumorinitiation properties and resistance to therapies. To evaluate a new concept of CSC theory and stemness origin of ATCs and PTCs, the present study assessed the cell lines that have subpopulations similar to CSCs.
Materials and Methods: Using MACS technique, cell positive for CD133 and/or CD44 were isolated and subsequently validated with flow Cytometry. Moreover, expression of some other stemness markers was evaluated.
Results: ALDH1A1 (ranged from 10 to 80% in four ATC cell lines, and 0 to 28% in two PTC cell lines), CD133 (ranged from 24 to 100% in three ATC cell lines and 16 to 78%), CD44 (about 83% and 100% in highly tumorigenic TPC1 and wt-BRAF cell lines) were significantly upregulated, while Nestin was downregulated in CD133pos and/or CD44pos subpopulations compared to CD133neg and/or CD44neg cells.
Conclusion: Numerous CSC markers have been identified to date, however stemness markers such as Sox-2, Oct-4 and ABCG2 were expressed in higher levels than previously been thought in PTC cell lines. In contrast to the previous studies, ATC and PTC thyroid cancers are similar to each other in terms of expression of CD44+ and CD133+ cancer stem cell lines.
Keywords: Cancer Stem Cell, ATC, PTC

Ps-41: Crosslink between Suppressed Endocrine Status, Failed PCNA-Related Hemostasis and Apoptosis in Testis of Nanomicelle Curcumin Received Rats

Moshari S[1*], Razi M[2], Nejati V[1], Najafi Gh[2], Minas A[2]

1. Department of Biology, Faculty of Basic Sciences, Urmia University, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: sana.moshari@yahoo.com

Background: Both nano and natural forms of Curcumin are known for their antioxidant, proapoptotic and antiproliferative effects. Thus, the curcumin is known as two edge sword as bioprotectant and biohazard agent. Considering proliferative characteristics of germ cells in male gonads, the nanomicelle

curcumin (NMCM) is suspected to adversely affect the male fertilization potential. Thus present study was conducted to illustrate the crosslink between endocrine potential, PCNA-related hemostasis and DNA integrity at germ cell level following exposure to different doses of NMCM.
Materials and Methods: Mature male rats were randomly divided into 4 groups (No in each group: 6) including: control, 7.5 mg/kg, 15 mg/kg and 30 mg/kg NMCM-received (orally by gavages) groups. Following 48 days, the Bax and Caspase-3 expression at mRNA and protein levels were evaluated by reversetranscriptase PCR (RT-PCR) and Immunohistochemistry (IHC) staining methods, respectively. The PCNA expression and DNA fragmentation were analyzed by IHC staining and DNA ladder, respectively. Finally Leydig cells distribution per one mm^2 of interstitial tissue and serum levels of testosterone were analyzed.
Results: The NMCM enhanced the Bax and Caspase-3 expression (especially in 30 mg/kg received group) at both mRNA and protein levels. Also the IHC analysis showed a significant reduction in PCNA protein levels in 30 mg/kg NMCM-received animals compared with control group. Furthermore, severe DNA fragmentation was observed in 30 mg/kg NMCM received group. More histological analyses revealed that, the NMCM reduced the number of Leydig cells per one mm2 of the interstitial tissue versus control animals. The serum testosterone was significantly ($P<0.05$) decreased in 30 mg/kg NMCM-received group.
Conclusion: Our data revealed that, the NMCM suppresses the testicular endocrine potential by reducing Leydig cell (as unique source of testosterone) number and eventually results in massive testosterone withdrawal. There after, diminished testosterone in turn triggers the apoptosis pathway via indirectly promoting Bax and Caspase-3 overexpression. In respect, the NMCM by reducing expression of PCNA, as main protein involving in DNA repairmen and/or replication, accelerates the testosterone withdrawal induced apoptosis by inhibiting DNA repairmen/replication.
Keywords: Nanomicelle Curcumin, Testosterone, Apoptosis, PCNA, DNA fragmentation

Ps-42: NLRC3 and NLRC5 Gene Expression Levels in IFN-Γ Treated Mesenchymal Stem Cells from The Wharton's Jelly of Human Umbilical Cord

Nahumi A[1*], Pirdel L[2]

1. Department of Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Faculty of Medical Sciences, Ardabil Islamic Azad University, Ardabil, Iran
Email: lpirdel@iauardabil.ac.ir

Background: Human umbilical cord Wharton's jelly has provided a new source for mesenchymal stem cells (MSCs). The highly proliferative capacity with low immunogenicity and multidifferentiation potential of its stem cells make them applicable for transplantation purposes. The NOD like receptors (NLRs) plays a variety of roles in antigen presentation of pathogens and damaged cells to suppress and/or modulate inflammation. In this study, the expression level of NLRC3 and NLRC5 genes were analyzed, and compared in both untreated and IFN-γ treated Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) which may represent an aspect of the INF-γ treatment in diminishing the inflammatory behavior of MSCs.
Materials and Methods: We successfully isolated MSCs from human umbilical cord Wharton's jelly using standard tissue culture. The expression of NLRC5 and NLRC3 genes were analyzed in untreated (control) and 24 hours of treatment with IFN-γ in WJMSCs using quantitative Real Time PCR.
Results: It was found that cell treatment with IFN-γ leads to a statistically significant increase of NLRC3 and NLRC5 gene expression ($P \leq 0.05$).
Conclusion: It seems that higher expression of NLRC3 and NLRC5 genes in the treated WJ-MSCs may make them a proper candidate to be used as a source for cell therapy in inflammatory conditions.
Keywords: Mesenchymal Stem Cells, Wharton's jelly, IFN-γ, NLRC3, NLRC5

Ps-43: Effect of Harmalin on Hypomethylation of Promoter Gene of P15 in Leukemic Cell Line NB4

Nikkhoo P[1*], Oodi A[2], Sotoodeh Nejadnematalahi F[1]

1. Department of Biology Genetics, Cellular and Molecular Group, School of Basic Science, Science and Research Branch of Islamic Azad University, Tehran, Iran
2. IBTO bldg, High Institute for Education and Research in Transfusion Medicine, Blood Transfusion Research Center, Tehran, Iran
Email: a.oodi@ibto.ir

Background: DNA methylation followed by tumor suppressor gene repression shows a critical role in the leukemia development. P15 is a cell cycle regulatory protein that works as a negative regulator of the cell cycle. Hypermethylation in the promoter of genes alleviate expression of these cell cycle control proteins which leads to increase the risk of cancerous cells. 5 –aZacitidine has been used in the treatment of malignancies such as cancers. Because of side effects and restrictions on the use of 5 –aZacitidine, synthetic nonnucleoside inhibitors such as Procainamide go to the side, although such inhibitors show poor hypomethylation power. Considering the limitations, usage of natural ingredients which effect on DNA hypomethylation, is of great concern. For example, curcumin, apple polyphenols notable examples of this huge resource, which in addition to being free of limitations and side effects of synthetic inhibitors. Curcumin has hypomethylation mechanism and impact on gene expression and leads to induction of P15. Harmalin, a beta carboline alkaloid derivative of peganum Harmalin, had shown antiproliferative effect on leukemic cell line.
Materials and Methods: This study aimed to measure the effect of Harmalin on hypomethylation promoter gene P15. Cell proliferation and cell cycle analysis were studied in NB4 cell line after treatment with Harmalin for 72 h. Tumor suppressor gene hypomethylation and reactivation was evaluated via MSP analysis and also real time PCR.
Results: Harmalin reduced cell proliferation in NB4 cell line in a time and dosedependent manner. Amount of cells in G1 phase of the cell cycle was increased by 15µg/ml of Harmalin ($P<0.05$). Antiproliferative doses of Harmalin induced hypomethylation and reactivation of P15 tumor suppressor promoter.
Conclusion: Our data indicate that Harmalin can be considered as a potential treatment for AML (Acute Myeloid Leukemia) and future studies are required to investigate the clinical efficacy of Harmalin, whether Harmalin can be employed as a single agent or as an adjuvant for AML treatment.
Keywords: Harmalin, Leukemia, p15

Ps-44: Effect of Temozolomide and Tranilast on Glioblas-

toma Multiforme Cell Line Apoptosis Related Genes

Pazhouhi M*, Khazaei M

Fertility and Infertility Research Center, Kermanshah University Of Medical Sciences, Kermanshah, Iran
Email: mona.pazhouhi@gmail.com

Background: Glioblastoma multiforme (GBM) is one of the most lethal forms of human cancer and temozolomide (TMZ) is currently part of GBM standard treatment. Combination therapy can enhance the anticancer activity of TMZ. Tranilast is an approved antiallergic drug. Previous studies have showed the antiproliferation ability of tranilast. The aim of the present study was to investigate the effect of TMZ and tranilast on apoptosis pathway in the human GBM cell line (U87MG).
Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS. The effect of TMZ and/or tranilast on the expression level of apoptosis related genes (Bax, Bcl-2, p53 and caspase 3) was analyzed by Real Time PCR. Total RNA from U87MG cells, treated with TMZ and/or tranilast for 72 h, was extracted. Complementary DNA (cDNA) synthesis was carried out and real time PCR was performed. The effect of TMZ and tranilast on U87MG nitric oxide production was evaluated by colorimetric Griess assay. Data were analyzed by oneway ANOVA and $P<0.05$ was considered significant.
Results: p53 was upregulated by TMZ, tranilast, and combination of both, as compared to untreated cells. Results showed downregulation of Bcl-2 and upregulation of Bax expression at 72 h treatment. Bax to Bcl-2 ratio was increased in U87MG cells treated with TMZ, tranilast and combination both. Expression level of caspase 3 was evaluated by TMZ, tranilast and combination treatment. U87MG cells treatment with TMZ and tranilast as a single treatment for 72 hr significantly decreased NO production. The minimum NO levels were observed as an effect of combination treatment.
Conclusion: TMZ and tranilast induced apoptosis and decreased NO production in U87MG cell line.
Keywords: Drug Combination, Glioblastoma Multiforme, Temozolomide, Tranilast

Ps-45: Effect of IFN-γ-treated Wharton's Jelly Mesenchymal Stem Cells on Nitric Oxidemediated Proliferation Suppression of Peripheral Blood Mononuclear Cells

Pirdel L[1*], Nahomi A[2], Safajoo M[2]

1. Department of Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Faculty of Medical Sciences, Ardabil Branch, Islamic Azad University, Ardabil, Iran
Email: lpirdel@iauardabil.ac.ir

Background: Wharton's jelly derived stem cells (WJMSCs) are stated to have higher expansion potential and low immunogenicity. The immune suppressive behavior of MSCs can be influenced in an inflammatory setting, resulted in lymphoproliferation suppression. Nitric oxide (NO) produced by MSCs is suggested to be the contributory factor in inhibition of T cell proliferation. We therefore investigated whether WJMSCs primed with proinflammatory stimuli, interferon-γ (IFNγ) can produce NO and whether NO is involved in their ability to suppress proliferation of peripheral blood mononuclear cells (PBMCs).
Materials and Methods: MSCs from human umbilical cord Wharton's jelly were isolated, cultured; MSCs from human umbilical cord Wharton's jelly were isolated, cultured, and characterized by flow cytometry. Mitomycin Ctreated MSCs were primed with IFN-γ. Next, primed and unprimed WJMSCs were co-cultured with allogeneic PHA- activated PBMCs at different ratios. The proliferation was assessed by MTT assay. NO levels were then determined in culture supernatants using the Griess reagent. Similar co-culture and assessment was set up even with primed and unprimed MSC without mitogenic stimulation.
Results: The proliferation of PBMCs was dose dependent as its rate in primed WJMSCs was lower as compared to unprimed WJMSCs without mitogenic stimulation while no significant difference was shown between primed and unprimed MSC in others. Mitogen induced proliferation response upon co-culture with primed WJMSCs was suppressed to a greater extent compared with unprimed WJMSCs. Comparison of NO production levels was shown a significant difference between primed WJMSCs co-cultured with PHA-treated PBMCs as compared with unprimed WJMSCs. In WJMSCs either primed or unprimed with IFNγ co-cultured with PBMCs without PHA stimulation, a slight increase in NO production was observed, but not dramatic relative to PHA mitogen stimulation. Therefore, additional experiments are required to investigate other molecular mechanisms.
Conclusion: These results suggest that NO may be one of the mediators of T-cell suppression by MSCs in a dosedependent manner under IFN-γ treatment.
Keywords: Wharton's Jelly, Mesenchymal Stem Cells, IFN-γ, Nitric Oxide, Immunosuppression

Ps-46: Effect of Interferon-γ on CD39 and CD73 Gene Expression in The Human Umbilical Cord Derived Wharton's Jelly Mesenchymal Stem Cells

Pirdel L[1*], Rahimzadeh M[2]

1. Faculty of Medical Sciences, Ardabil Branch, Islamic Azad University, Ardabil, Iran
2. Department of Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Email: lpirdel@iauardabil.ac.ir

Background: Human umbilical cord Wharton's jelly mesenchymal stem cells (WJMSCs), which have high proliferative capacity with low immunogenicity, has provided a new source for regenerative medicine. Adenosine is a strong immunosuppressant that acts mainly through its receptor A2a (ADORA2A). The ectonucleotidases CD39 and CD73 play an important role in the conversion of ADP/ATP to AMP and AMP to adenosine, respectively. In this study, Changes in the expression of CD39 as well as CD73 were evaluated in WJMSCs either primed or unprimed with proinflammatory cytokine IFN-γ which may be correlated with the suppressive function of MSCs accompanied by increased production of adenosine.
Materials and Methods: We successfully isolated MSCs from human umbilical cord Wharton's jelly and cultured with or without IFN-γ. The expression of CD39 and CD73 genes was analyzed in WJMSCs either primed or unprimed with IFNγ after 24 h using quantitative Real-time PCR.
Results: It was found that IFN-γ treatment leads to a statistically significant increase of CD39 gene expression ($P\leq 0.05$) while there is no significant difference in CD73 gene expression

between primed and unprimed WJMSCs.
Conclusion: The results suggest that the modulation of components related to adenosine signaling such as CD39 in the treated WJ-MSCs may, in part, be contributed to some of the immunomodulatory behavior of MSCs in an inflammatory setting.
Keywords: Wharton's jelly, Mesenchymal Stem Cells, IFN-γ, CD39, CD73

Ps-47: Exosome Nano Exchanging Carriers for Cellular Trading: A Decision Knowledge Microenvironment Approach

Rady Raz N*, AkbarzadehT MR

Department of Computer and Electrical Engineering, Center of Excellence on Soft Computing and Intelligent Information Processing, Ferdowsi University of Mashhad, Mashhad, Iran
Email: radyraz@mail.um.ac.ir

Background: The best place for detection, treatment, and prevention of different diseases with more precision and less noise is their own microenvironments. The microenvironment approach leads to a dynamic localglobal vision using fewer intermediate processes and results in more decision knowledge. The synergy of nano technology and artificial intelligence promises to shed light on this approach, and brings out the bright dreams into the hands of labs. In this paper we propose an in silico nano delivery system for cellular exchanging. Here engineered exosomes which are nontoxic, nonimmunogenic, and capable for doing targeted delivery in challenging situation such as Blood Brain Barrier (BBB) are introduced as nano carriers. Many researches have been done regarding cell free therapy using exosomes in responding to tissue injury, infection, and disease. Mesenchymal stem cells (MSCs) derived exosomes are famous in using for cell free regenerative medicine. They can separate from blood and flow toward definitive target due to superparamagnetic behavior and strong response ability to external magnetic field. Exosomes are used for cellular communication, immune system suppression, growth regulation, metastasis, doing different cellular cargo delivery. These biological vesicles are suitable for loading multiple drugs while they are capable of protecting contents from degradation, in contrast to their competitors such as viruses, liposomes, nanoparticles which have nununiform particle size, agglomerates tendency, and a quick clearance by the reticuloendothelial system.
Materials and Methods: In this paper using a decision knowledge microenvironment approach, we propose an in silico nano delivery system for cellular exchanging. Here engineered exosomes which are nontoxic, nonimmunogenic, and capable for doing targeted delivery in challenging situation such as BBB are introduced as a nano carriers. Using proper disease markers, they will be secreted in the irregular microenvironments for therapy.
Results: An in silico exosome nano delivery system based on microenvironment approach and decision knowledge for cellular exchanging improves the cancer microenvironment.
Conclusion: Nano technology provides a wide range of applications in cellular therapy. In a case of cellular disease like cancer, microenvironment's investigation is a great help in detection, treatment, and prevention. Toward this aim, a great wealth of literature is done in order to engineer nature for therapy. In this paper, cellfree therapy is completely reviewed and then an in silico nano delivery system for cellular exchanging based on exosomes is presented.
Keywords: Nano Delivery, Exosome, Decision Knowledge, Cell free Targeted Therapy

Ps-48: Meta-Analysis of Micrornas And their Potential in Regulation of Self-Renewal in Breast Cancer Stem Cells

Rahimi M[1, 2*]

1. Medical Biotechnology, Advanced Medical Sciences, Tabriz University of medical science , Tabriz , Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: rahimi.mahsa69@gmail.com

Background: Cancer stem cells (CSCs) have different role in tumor development including tumor growth, resistance to drugs and metastasis. Recently, CSCs are widely accepted as novel targets for anticancer treatments. Recent progress has highlighted the significant role of miRNAs in controlling the Stemness in CSCs. Therefore, the present Meta-analysis based on literature mining and bioinformatics approaches was performed to find the miRNAs which regulate Stemness potential in breast cancer stem cells.
Material and Methods: Literature mining and different sets of data on miRNAs expression profiling were searched using the following keywords: "Breast Cancer, Stem Cell, microRNA, Chemoresistance or Drug Resistance, Metastasis or EMT". Studies were excluded from the analysis if: (i) the review articles or letters, (ii) studies with insufficient data. Then, we highlighted the most frequent miRNAs which regulated the stemness genes. In addition, we extracted studies that used quantitative RT-PCR and pooled them together by using metadisc software.
Results:A total of 322 articles were yielded after the literature searching. After fulltext reviewing, a total of 143 miRNAs were found to be expressed in breast cancer cells; 88 of them were dysregulated in breast cancer stem cell (49 up regulated and 39 downregulated). Interestingly 9 of them including miR-34a, miR-21, miR-373, miR-200c, miR-10b, miR-520c, miR-335, miR-30c and miR-204 were found to regulate both stemness and metastasis in breast cancer stem cells.
Conclusion: The same miRNAs in breast cancer stem cells regulate stemness and metastasis, that may be addressed as diagnostic targets or used as therapeutics agents..
Keyword: Breast Cancer, Cancer Stem Cells, microRNAs, Metastasis, Stemness

Ps-49: Gene Regulatory Interactions in Mouse Embryonic Stem Cells and Inner Cell Mass

Rasooli P[1, 2*], Hajseyed Nasrollah ZS[3], Khosravi P[4], SharifiZarchi A[1, 5], Totonchi M[1, 6]

1. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
2. Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), University Hospital Cologne (UoC), Cologne, Germany
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. School of Biological Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
5. Department of Computer Engineering, Sharif University of Tech-

nology, Tehran, Iran
6. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: m.totonchi@royaninstitute.org

Background: Mouse embryonic stem (ES) cells can be derived from inner cell mass (ICM) of day 3.5 mouse blastocyst under various pluripotency conditions. Since ES cell originates from ICM, it is considered as *in vitro* counterpart of ICM and the similarities between them are obvious, such as their differentiation potential. Mouse ES cells like ICM can give rise to all the fetal tissues if transplanted in the embryo. It has been reported that they have differences in some critical features including selfrenewal. A better understanding of ICM and ES cell similarities and differences can be achieved via investigating into ES cell derivation process. Recently a highly efficient culture condition for ES cell isolation has been devised, named R2i, that provides an opportunity for dissecting molecular mechanisms underlying this process. Timeseries experiments are important to comprehend dynamic biological processes. One of the most popular types of data that can be measured over time in these experiments is timeseries gene expression data. A powerful method for analyzing such data is the usage of gene regulatory networks (GRN) and that is discovering regulatory interactions between genes. GRNs can model the regulatory effect of a transcription factor on its target gene. Two parts of a network are nodes and edges. In a GRN, genes are the network's nodes and each edge represents an interaction between the two genes. Modeling biological systems can be helpful in reducing the cost of experimental procedures and provides holistic insight about a living system.
Materials and Methods: Here we generated statespecific GRN for different time points during ES cell derivation using previously generated timeseries gene expression data. Interactions extracted from a comprehensive mouse GRN.
Results: With analyzing and comparing ICM specific and ES specific networks we highlighted the specific gene interactions in ICM and ES cells.
Conclusion: Our ES cell derivation model led us to perceive the similarities and differences between ICM and ES cell and provided more details about this cell fate conversion.
Keywords: Mouse Embryonic Stem Cell, Inner Cell Mass, Gene Regulatory Network, Time-Series Gene Expression Data

Ps-50: Is Gastro Spheres Driven Regulatory T Cells *in vitro*?

Rezalotfi A[1, 2*], Solgi Gh[1], Ebrahimi M[2]

1. Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Immunology Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
Email: Mebrahimi@royaninstitute.org

Background: Regulatory T (Treg) cells determinate by CD4+CD25+FOXP3+ cells, have immune suppressive effect and are prominent with tumor progress. Among tumor cells, a rare population with pluripotencies mediated immune suppressive effects is responsible for the most tumor progression. Although Treg cells in the most advanced solid tumors has been reported, but their interaction with gastro spheres have been elusive, depending on tumor type or site. The object of this study is to find the effect of gastro spheres on differentiation of Treg.
Materials and Methods: Gastro spheres were used as a model for enriching cancer stem cell. CD4+CD25+-FOXP3+ cells were assessed by flow cytometery analysis in peripheral blood mononuclear cells (PBMCs) from a normal individual, which were treated with MKN-45 cells, a human gastric cancer cell line, and their gastro spheres directly and indirectly. Both experiments were performed in presence of IL-2 as T cell activator for 6 continues days. The expansion rate of treated T cells in groups was evaluated by CFSE and phenotype of Tregs by flow cytometery.
Results: Flow cytometery analysis revealed that there was a decrease in percentage of CD4+CD25+FOXP3+ Tregs in PBMCs were treated with gastro spheres and its condition medium compared with nongastro spheres and its condition medium (almost 6% vs. 7%). It was also demonstrated that T cell expansion was increased in gastro spheres but decreased in its condition medium treatment compared with the control group (about 3 fold) (P value<0.05).
Conclusion: We conclude that gastro spheres and its condition medium reduces the Tregs population. The effect of CSC on T cell expansion was various. When gastro spheres directly had contact, promoted T cell expanse, but their soluble factors reduced T cell proliferation.
Keywords: Regulatory T cells, Gastro Spheres, Gastric Cancer Stem Like Cells, Tumor Microenvironment

Ps-51: Actual Reason of Tau Toxicity upon Neurodegeneration

Roqanian Qazvini Sh[1, 2*], Shahpasand K[2], Ahmadian Sh[1]

1. Department of Biochemistry, Institute of Biochemistry and Biophysics, Tehran, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Emails: shahpasand09@gmail.com, sh.ahmadian@ut.ac.ir

Background: Tau malfunction has been observed in several neurodegenerative disorders commonly named tauopathies. Recently it has been shown that phosphorylated tau at Thr231 in the cis conformation is the central mediator of cell death upon tauopathy but it remains obscure that how cis ptau kills the cells. We have found that neurotoxic cis ptau initially moves into nucleus causing cell death. Thus, we hypothesized that cis p-T231-tau is becoming neurotoxic upon its nuclear translocation.
Materials and Methods: To test this hypothesis, we have performed immunoflourescent staining and apoptosis assay on primary cultured neurons and traumatic brain injury mice models.
Results: Our results showed that neurotoxic cis, but not trans pT231-tau moves into nucleus in stressed out neurons in a timedependent manner. Moreover, translocated cis ptau causes neural cell death
Conclusion: We have shown that cis ptau plays its pathogenic roles upon its translocation and concluded that tau kills the cells through interrupting some physiological processes by interacting with nuclear factors. Our data open new windows toward tauopathies molecular mechanisms and would help us determine promising therapeutic strategies against the devastating disorders.
Keywords: Cis ptau, Tauopathy, Neurodegeneration, Nuclear

Translocation

Ps-52: Resveratrol Did Not Modulate The Apoptotic Rate of Human Embryonic Stem Cells

Safaeinejad Z[1*], Nabiuni M[2], Ghaedi K[3, 4], Peymani M[4, 5], Nasr Esfahani MH[4]

1. Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
3. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
4. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
5. Department of Biology, Faculty of Sciences, Sharekord Branch, Islamic Azad University, Shahrekord, Iran
Email: safayinejad@yahoo.com

Background: Embryonic stem cells (ESCs) can undergo unlimited selfrenewal and retain the ability to differentiate into any cell type in the body. Human ESCs (hESCs) hold great promise for understanding early human embryonic development and generating clinically useful cell lines. In view of this a great deal of effort is focused on experiments aimed at improving hESCs survival. Resveratrol (RSV) or trans-3, 5, 4-trihydroxystibene is naturally antifungal phytoalexin, existing in the skins of red grape and a variety of other plant species. Although extensive *in vitro* and animal researches have shown that RSV possesses a wide range of biological properties, there is not any study that examined the effect of this substance on survival of hESCs. Therefore, this study was performed to clarify the role of RSV on hESCs survival.
Materials and Methods: HESC line, RH6 was used in present study. Cells were cultured on matrigel and fed daily with specific supplemented hESC medium. Cell apoptosis was examined using flowcytometry for annexin Vpositive cells. Effect of RSV on proapoptotic gene BAX and antiapoptotic genes BCL-2 as well as BCL-XL was evaluated by realtime quantitative polymerase chain reaction (RT-qPCR).
Results: RSV did not exert any significant change in the rate of apoptosis, as there was no significant change in the percentage of annexin Vpositive cells. RT-qPCR data showed that antiapoptotic genes up regulated in the presence of RSV.
Conclusion: We concluded that RSV did not modulate hESCs cell viability but elevated antiapoptotic genes expression
Keywords: Human Embryonic Stem Cells, Resveratrol, Apoptosis

Ps-53: Evaluation of NLRP6 and NLRP12 Gene Expression in Human Wharton's Jelly Mesenchymal Stem Cells Treated With IFN-Γ

Safajoo M[1*], Pirdel L[2]

1. Department of Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Faculty of Medical Sciences, Ardabil branch, Islamic Azad University, Ardabil, Iran
Email: lpirdel@iauardabil.ac.ir

Background: Human umbilical cord Wharton's jelly is considered as a novel source of mesenchymal stem cells (MSCs) with proliferative and multidifferentiation capacities. Having a negligible immunogenicity, these stem cells are regarded as proper candidate to be used for transplantation modalities. Despite lacking a clear mechanism, MSCs with their potential in suppressing the immune responses are extensively studied for several clinical applications. We aimed to clarify the mechanisms beyond the suppressive effect of MSCs in primary MSC culture. Having a variety of functions during antigen presentation of pathogens and immunemodulatory effects, the NOD like receptors (NLRs) were among our focus. We measured the expression levels of NLRP6 and NLRP12 genes before and following the treatment with IFN-γ in Wharton's Jel-ly-Mesenchymal Stem Cells (WJ-MSCs) to assess whether these NLR members are involved in MSC-mediated immunemodulation.
Materials and Methods: MSCs were isolated from Wharton's jelly using tissue culture technique. Real-time quantitative PCR was used to measure the expression levels of NLRP6 and NLRP12 genes in untreated (control) and IFN-γ-treated WJM-SCs.
Results: It was shown that the cells treated with IFN-γ have an upregulation of NLRP12 gene compared to untreated cells while mRNA expression of NLRP6 failed to increase following IFN-γ treatment.
Conclusion: An upregulation of NLRP12 in WJ-MSCs treated with IFN-γ may suggest using this cytokine to modulate the immune responses against transplanted cells in inflammatory conditions.
Keywords: Wharton's jelly, Mesenchymal Stem Cells, IFN-γ, NLRP6, NLRP12

Ps-54: Cytotoxicity of TiO2 Nanomaterial in Sunscreen on Stem Cells

Sarikhani M[*], Alizadeh E, Deylam M, Firuzamandi M, Hejazy M
Department of Pathobiology Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
Email: alizadehe@tbzmed.ac.ir

Background: Metal oxide nanoparticles have been used prevalently in different medical applications including sunscreens, cosmetics, vitamin coatings, toothpaste and so on. Particularly, Titanium dioxide(TiO) has a higher refractive index and strong white coloring. TiO has the potential for blocking out of both UVA and UVB. Therefore has a great convenience in sunscreens. In this way, the TiO nanoparticles are in near contact with external layers of body cells. The aim of this study is to evaluate the possible toxic effects of TiO nanoparticles on rat adipose/ bone marrow derived stem cells.
Materials and Methods: In the present study, mesenchymal stem cells were isolated from adipose (rAMSCs) and bone (rBMSCs) marrow of 3 week rat. The routine DMEM medium enriched with fetal bovine serum was used for expansion of rAMSCs or rBMSCs . In the next step the rAMSCs or rBMSCs subjected to CD markers analysis by flow cytometry. Multipotency of each group were analysed by osteogenic or adipogenic differentiation. TiO in different concentrations(1 to 320 µg/ml) were added to each well of 96-well plates and MTT assay was performed after24, 48, 72, 96 hours incubation.
Results: Our results showed that either rBMSCs or rAM-SCs showed excellent differentiation potential. The MTT assay showed that TiO can cause dose and time dependent effects on viability of stem cells. TiO was safe in concentration ranges less than 240, 170, 150, 70 µg/ml after 24, 48, 72, 96 hours

for rAMSCs. But in rBMSCs the cells showed more sensitivity and the maximum cytotoxic doses are 200, 100, 50, 10 µg/ml respectively.
Conclusion: Our results imply that at higher doses and long incubation periods TiO can cause cytotoxic effects on MSCs.
Keywords: Titanium Dioxide Nanoparticles, Rat MSCs, Cytotoxcity

Ps-55: Human Chemical Induced Neuronal Cells as An Efficient Toll for Investigating Neurodegeneration Process in Familial Alzheimer's Disease

Shahbazi S[1, 2*], Habibi Reza M[1], Shahpasand K[2]

1. Department of Cell and Molecular Biology, Faculty of Biology, University of Tehran, Teheran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: shahpasand09@gmail.com

Background: Neurons generated from somatic cells are being considered as an efficient model for examining several neurological disorders *in vitro* especially in those with Familial types. Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to gradual cognitive decline and is classified into early (EOAD) and late onset (LOAD) forms with incidence of 2% and %98 of the patients respectively. EOAD usually follows an autosomal pattern and resulted from mutations in a single gene and thus considered as Familial AD. Despite of extensive considerations AD molecular mechanisms and genes mediating its process have remained obscure thus far. There are two major pathological hallmarks playing part in AD including tau hyperphosphorylation and Amyloid precursor protein misprocessing. Tau is moderately being phosphorylated under physiological conditions but its hyperphosphorylation would result in its pathogenicity . It has been reported that phosphorylated tau at Thr231(pT231-tau) exists in the two distinct cis and trans conformation , for which trans is physiologic but cis is pathogenic; playing its roles at the very early stages of tauopathy. We have reprogrammed primary human fibroblasts from control healthy and AD patients in to mature neurons and examined cis ptau formation potency.
Materials and Methods: Stressed out neurons of either group underwent immunofluorescence analysis and western blot for cis ptau detection. MitoTracker Green FM was used to investigate the axonal transport in those neurons.
Results: APP, PSEN1, PSEN2 and APOE genotypes were determined by sequencing analysis of primary human fibroblast in either group . We have shown that control and FAD neural cells differ not only in cis ptau production, but also in mitochondrial transport demonstrating of disrupted axonal microtubule networks.
Conclusion: Taking these together, our results suggest that hciN cells transdifferentiated from FAD patient can recapitulate the neuropathological processes of the disease, and cis ptau is the early driver of neurodegeneration upon AD. Thus, we believe cis mAb targeting and neutralizing cis ptau sounds like an efficient therapeutic agent against the devastating disorder.
Keywords: hciN Cells, Familial Alzheimer's Disease, Cis P-Tau, Early Driver of Neurodegeneration

Ps-56: Synergistic Effect of Teucrium Polium Extract and Tranilast on Human Umbilical Vein Endothelial Cell

Sheikhbahaei F[1*], Khazaei M[1], Nematollahi Mahani S[2]

1. Kermanshah University of Medical Sciences, Fertility and Infertility Research Center, Kermanshah University, Kermanshah, Iran
2. Kerman University of Medical Sciences, Physiology Research Center, Kerman University, Kerman, Iran
Email: asalpt80@yahoo.com

Background: Tranilast, an antifibrotic drug has shown promising antitumor activities through inhibition of angiogenesis. Teucrium polium (TP) is a member of Lamaceae family with antitumoral properties . The aim of the present study was to investigate the cotreatment effects of tranilast and TP on Human umbilical vein endothelial cells (HUVEC) as a model of *in vitro* angiogenesis .
Materials and Methods: The HUVEC line was treated with different doses of Tranilast and/or TP. The cell cytotoxicity was assessed by LDH assays. The effect of TP and/or tranilast on the expression level of apoptosis related genes (Bax, Bcl2) was analyzed by Real Time PCR. Total RNA from HUVEC cells, treated with TP and/or tranilast for 72 hr, was extracted. Complementary DNA (cDNA) synthesis was carried out and real time PCR was performed. The effect of TP and tranilast on HUVEC nitric oxide production was evaluated by colorimetric Griess assay. Data were analyzed by oneway ANOVA and $P<0.05$ was considered significant
Results: Tranilast and TP significantly decreased survival rate of HUVEC cells. However, Cotreatment of TP and Tranilast significantly decreased viability compared to singleagent treatment. In addition, NO production decreased significantly by HUVEC . Furthermore, Bax and Bcl-2 expression was significantly affected by TP and/or tranilast and cotreatment. A significant increase in bax and a decrease in bcl-2 mRNA expression was detected in cotreated group.
Conclusion: TP and tranilast induced apoptosis and decreased NO production and survival rate in HUVEC cell line
Keywords: Angiogenesis, *In vitro* Angiogenesis, Teucrium Polium, Tranilat, Apoptosis

Ps-57: Glioblastoma Stem Cells and its Impact on Drug and Radioresistance

Shoja M*, Ghaniyan F, Montazeri AS, Nazeriye F, Hoseinzade H

Department of Radiology, Faculty of Paramedicine, Semnan University of Medical Sciences, Semnan, Iran
Email: moh3n_sh_66@semums.ac.ir

Background: Glioblastoma multiforme (GBM) is aggressive cancer and malignant tumor in the brain, causing a large mortality in adults. The prevalent standard treatment is Surgical followed by radiotherapy and chemotherapy agent temozolomide but increasing the survival of patients is one of the challenges for oncologists. Overcoming this barrier is a significant objective, even if difficult to achieve, because our understanding of GBM Stem Cells (GSCs) and their microenvironmentdependent pathways of therapeutic resistance is still limited. Thereby molecular targeting of GSCs may directly improve current therapies efficacy.
Materials and Methods: In glioblastoma stem cells L1CAM mediated signaling makes radioresistance, is a therapeutic target for GBM therapy. GSCs express a variety of drug resistance proteins like MGMT and antiapoptotic genes such as

FLIP, BCL-2, BCL-XL and cIAP1. Also GSCs stimulate tumor angiogenesis by expressing elevated levels of VEGF and contribute to tumor growth. On the other hand, hypoxic condition induced VEGF expression, which has been translated into a useful therapeutic strategy in the treatment of recurrent or progressive GBMs.
Results: Hypoxia may enhance tumor progression and therapeutic resistance through its promotion of a cancer stem cell phenotype and induction of VEGF and other proangiogenic factors. Cancer cells in each tumor has greater potential of cancer initiation and repopulation. GSCs are relatively resistant to radiation due to preferential response of the DNA damage checkpoint and the enhanced DNA repair capacity and likely are responsible for GBM tumor recurrence.
Conclusion: In this review, we summarize the current understanding and advances in glioma stem cell research, and discuss potential targeting strategies for future development of antiGSC therapies.
Keywords: GBM, Radiation, Resistance, Cancer, Stem Cell

Ps-58: Expression Level of Transforming Growth Factor-β1 Is Reduced by Increasing The Passage Number of Equine Mesenchymal Stem cells

Shojaee A[1], Parham A[1, 2*], Ejeian F[3], Masaeli E[3, 4], Nasr Esfahani MH[3]

1. Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Embryonic and Stem Cell Biology and Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
4. Department of Textile Engineering, Isfahan University of Technology, Isfahan, Iran
Email: parham@um.ac.ir

Background: It is widely accepted that growth factors (GFs) powerfully regulate the biological responses of mesenchymal stem cells (MSCs). TGF-β has been attracting attention in the tenogenic regenerative field as it participates in all phases of tendon healing process. Moreover, TGF-β involves in tendon differentiation of MSCs. Since passage number influence function and behavior of the cells as well as gene expression profile, this study was aimed to investigate mRNA expression of TGF-β1 in equine MSCs at different passages.
Materials and Methods: Equine MSCs were isolated from the fat tissue of gluteal region and expanded. The cells at passages 5 and 8 were seeded and maintained in basic culture medium. 75 % confluency of cels was considered as day 0 and cells were harvested at days 1 and 3. Then, total RNA was extracted and transcribed into cDNA using commercially available kits. Primers for GAPDH (as internal control) and TGF-β1 were designed. Quantitative RT-PCR (qPCR) was performed using a SYBR green master mix and data was analyzed using Student t test.
Results: The mRNA expression of TGF-β1 at P5 and P8 of equine MSCs were compared at 1 and 3 days after 75% confluency. Our data revealed that TGF-β1 expression was significantly higher at P5 compared with P8 in day 1, but there was no significant difference at day 3.
Conclusion: Based on the results, the expression level of TGF-β1 is significantly affected by the cell passage number of equine MSCs. So, tenogenic differentiation capability of quine MSCs can be affected by passage number which should be considered in tenogenic differentiation experiments.
Keywords: Mesenchymal Stem Cells, TGF-B1 Expression, Passage Number, Equine

Ps-59: Identification of Transcription Factors Involved in Conversion of PC12 Cells into Neuron-Like Cells by Staurosporine

Soleimani T*, Fallahi H, Azadbakht M

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: Neurogenesis is an important process occurs during embryogenesis in all animals. Neurons are generated from neural stem cells and progenitor cells. Stem cells exhibit two characteristics, selfrenewal and capacity for generating almost any types of differentiated cells. In adult mammals, neurogenesis occurs in three places in the brain, including dentate gyrus, the subventricular zone (SVZ) and the olfactory bulb. Impaired adult neurogenesis was demonstrated in many degenerative diseases, such as Alzheimer. Neural cells can be generated from somatic cells through somatic cell reprogramming or via direct conversion. There are a variety of cell lines, which are used as models for the study of neural differentiation. Induced cell differentiation has been studied in retinal ganglion cells (RGCs), PC12 and SH-SY5Y. PC12 cell line derived from a pheochromocytoma of the rat adrenal medulla, have been widely used as a preferable model for neural differentiation because this cell line has advantages beyond others including showed no recognizable changes in properties for the first 70 passages in culture. This cell line can be differentiated by several chemicals and transcription factors such as nerve growth factor (NGF), epidermal growth factor (EGF), Rapamycin, Staurosporine (STS), trimetyltin (TMT), Ginsenoside-Rd and pituitary adenylate cyclaseactivating polypeptide (PACAP). Staurosporine is a protein kinase inhibitor, which induces neurite outgrowth in PC12 cell lines within a few hours in comparison other materials. Many studies showed that transcription factors could convert somatic cells into neuron-like cells. However, the complete molecular significance of these factors on this cell line has not been uncovered.
Materials and Methods: In this study, the impact of the staurosporine as an inducer of neural differentiation in PC12 cell line was investigated. PC12 cells were grown in RPMI 1640 culture medium, supplemented with 10 % fetal bovine serum (FBS). After treatment with staurosporine we have extracted total RNA from cells. Following RNA-seq analysis, we have identified differentially expressed genes (DEGs). We then constructed and analyzed regulatory networks for transcription factors involved and their genes using JActivemadule, MCODE and Network Analyzer apps from the Cytoscape software environment.
Results: By analysis of RNA-seq data and using online tools and freely available software, we have identified the most important transcription factors (TFs). Network analyses showed integration of these TFs into core differentiation regulatory network.
Conclusion: Such studies would be useful in clarifying the molecular pathways of neural differentiation. We hope our analysis can facilitate the generation of neurons for *in vitro* disease mod-

elling or develop therapies to stable induction of neuron cells in the injured brain.
Keywords: RNA Sequencing, PC12 Cell Line, Staurosporine, Transcription Factor, Network Analysis

Ps-60: Dissecting MiRNAs Regulatory Network Involved Neurogenesis of PC12 Cells Induced by Staurosporine

Soleimani T*, Fallahi H, Azadbakht M

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: Understanding the transcriptome is necessary for explaining the functional elements of the genome and also for understanding development and disease. During recent developed many highthroughput sequencing assays have been developed such as RNA sequencing (RNA-seq). RNA-seq, which provides a precise measurement of transcripts. In the current study, we have used rat PC12 cell line derived from a pheochromocytoma, which used as an established model for studying neural differentiation. PC12 cell line can be differentiated into neuron-like cells by several chemicals and transcription factors including staurosporine. Staurosporine is a protein kinase inhibitor, which induces neurite outgrowth in PC12 cell lines, similar to other factors such as nerve growth factor (NGF), epidermal growth factor (EGF) and pituitary adenylate cyclaseactivating polypeptide (PACAP). Staurosporine can reprogram PC12 cell lines toward neurogenesis in about six hours therefore staurosporine is a quick and easy inducer. However, precise role of microRNAs during differentiation by staurosporine is unknown.
Materials and Methods: In the current work, PC12 cell line was maintained in RPMI 1640 supplemented with 10% FBS. Staurosporine were added to the culture medium, then we have extracted RNAs of induced neuron-like cell. After analysis of RNA-seq data, we found a list of differentially expressed genes (DEGs). To identify microRNAs, DE genes was introduced into Target scan miRNA data base. Then regulatory network was constructed using the Cytoscape software environment. Finally, we have identified the key hub microRNAs involved and their targets that have roles in the neural differentiation of PC12 cell line.
Results: After finding differentially expressed genes, by comparing the gene expression patterns between untreated and staurosporinetreated PC12 cell line, key miRNAs that targeting the list of genes has been identified and regulatory network corresponding these miRNAs were extracted.
Conclusion: Our analysis of RNA sequencing would be helpful in identification of hub miRNAs involved in neural differentiation. These findings could develop new protocols to induce neural cell differentiation.
Keywords: RNA Sequencing, PC12 Cell Line, Staurosporine, MicroRNA, Network Analysis

Ps-61: Identification of MicroRNAs Involved in Direct Conversion of Fibroblasts into Cardiomyocyte-Like Cells

Soleimani T*, Falsafi N, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Background: Today, the generation of cardiomyocyte-like cells from somatic cells, using different elements such as chemical cocktails, transcription factors and the combination of transcription factors and microRNAs (miRNAs), holds great potential for cell replacement therapy. These induced cardiomyocyte-like cells (iCMs) have global geneexpression profiles and many features, similar to cardiomyocytes. Since, one of the most causes of morality in the world is cardiovascular diseases (CVD), therefore finding new and suitable methods in order to stable induction of cardiomyocyte is required. To achieve this goal many scientists have been examining the effects of different factors such as chemical cocktails, Transcription factors (TFs) and the combination of TFs and microRNAs to convert different kind of fibroblasts into cardiomyocytelike cells. However, the effectiveness of cardiac reprogramming remains controversial issue and there is evidenced that indicates issues with direct conversions protocols. In the current study, we have used bioinformatics approaches to uncover the most important miRNAs involved in this conversion.
Materials and Methods: In the current study, we have combined data sets obtained from four independent GSEs. There have used different chemical cocktails or TF-miR combination as inducer. After finding differentially expressed genes (DEGs), this list of DE genes was submitted to the Enrichr website. Using online tools and freely available software, we constructed two independent networks for induced cardiomycyte-like cells by chemical cocktails or the combination of TFs and microRNA. Finally, we have compared network analysis of hub miRNAs between induced cardiomyocyte-like cells by chemical cocktails or the combination of TFs and miRNA.
Results: Our insilico approaches were able to identify the most important miRNAs in direct conversion of human or mouse fibroblasts into cardiomyocyte-like cells. Besides, the most affected pathways during this process were studied and key elements of the network were unveiled.
Conclusion: Our work demonstrates an appropriate method for identification of the key microRNAs involved in fibroblast reprogramming to cardiomyocyte-like cells. These could be useful in optimising current protocols to obtain cardiomyocyte-like cells for regenerative medicine.
Keywords: MicroRNAs, Regenerative Medicine, Induced Cardiomyocyte-Like Cells, Network Analysis

Ps-62: Investigation of miR-23 and miR-27 Influence on Regulation of Tumor Suppressor Gene CFIm 25 in Human Breast Cancer Cell Line

Tamadon M[1*], Kouhkan F[2]

1. Department of Biology, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran
2. Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
Email: f.kouhkan@yahoo.com

Background: Global incidence of breast cancer according to the National Cancer Institute highlights the importance of focusing as much as possible on developing treatment strategies. Just recently, microRNAs (miR) are emerging as biomarkers and potential therapeutic targets in tumor management. Human Cleavage Factor Im (CFIm) is an essential component of the pre-mRNA 3′ processing complex that functions by the regulation of poly(A) site selection through the recognition of UGUA sequences upstream of the poly(A) site. Recently CFIm25 has been identified as a broad repressor of proximal poly(A) site usage that, when depleted,

increases cell proliferation. In this area, we have previously suggested the possible role of some oncogenic microRNAs including miR-23 and miR-27 in regulation of tumor suppressor gene CFIm 25. So, the current study aimed to investigate the influence of miR-23 and miR-27 downregulation on CFIm 25 expression level in human breast cancer cell line and its proliferation.
Materials and Methods: Human breast cancer cell line of MDA was infected with lentiviruses containing either anti-miR-23 or anti-miR-27 precursor sequences. The RNA expression level of miR-23, miR-27 and CFIm25 were estimated in MDA infected cells by QRT-PCR. Protein level of CFLm25 genes in human MDA were also analyzed by western blotting.
Results: Real-time PCR showed that miR-23 and miR-27 knockdown enhanced CFIm25 expression level in MDA treated cells. Western blotting results also confirmed that downregulation of miR-23 and miR-27 in MDA cell lines dramatically enhanced CFIm25 expression.
Conclusion: Overall, this is the first study which evidently indicates miRNAs correlation with CFIm25 expression in breast cancer cells. We indicated that miR-23 and miR-27 overexpression might be one of the factors contributing to the cell proliferation in breast cancer through CFIm25 downregulation. So, our data suggests that oncogenic role for selected miRNAs and presents a rationale for the down regulation of these miRNAs as a novel strategy to improve treatment response in breast cancer.
Keywords: Breast Cancer, Microrna, Tumor Suppressor, CFIm25

Ps-64: MicroRNA as A Mediator of Omega 3 Fatty Acids in Ovine Adipocytes Metabolism: Effects on Lipid Accumulation and Oxidation

Veshkini A[1, 2*], Khadem AA[1], Mohammadi Sangcheshmeh A[1, 2], Assadi Alamouti A[1], Kouhkan F[2], Salehi A[1], Soleimani M[3]

1. Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Pakdasht, Tehran, Iran
2. Stem Cell Technology Research Center, Tehran, Iran
3. Department of Hematology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
Email: arash.veshkini@yahoo.com

Background: Lipid metabolism dysfunction in adipose tissue is in great association with obesity and related metabolic disorders can substantially interfere with health and life quality. Evidences from recent publications indicated the effect of Omega-3 fatty acids on lipid and glucose homeostasis, and adipogenesis through microRNA regulation of nuclear transcription factors within the adipose tissue. Therefore, the objective of the current study was to characterize molecular pathways that affect lipid metabolism in adipocytes following fish oil (source of Omega-3 PUFAs) treatment.
Materials and Methods: Ovine mesenchymal stem cells (MSCs) were induced to differentiate to adipocytes with with 0.5 mM hydrocortisone, 60 mM indomethacine, and 0.5 mM isobutylmethylxanthine for 21 days. Adipocytic markers were then evaluated using in situ Oil Red O (ORO) staining. Fish oil (50µM) was added to cultured media of the adipocytes for 48 h. Relative expression of miR-2368 and its target gene PPARα along with miR-142-5p and its downstream gene ACSL6 were evaluated by QRT-PCR.
Results: 21 days after differentiation, cytoplasmic fat vesicles in differentiated MSCs that were stained by ORO indicated successful adipocytes formation. After treatment, Real-time PCR specified no significant changes in the expression of miR-2368 although the expression of miR-142-5p was downregulated in fish oil group. Also, Fish oil treatment had no significant effect on the expression of PPARα, as a marker of accumulation and oxidation of lipids, but significantly increased the expression of lipid metabolism and oxidation marker, ACSL6.
Conclusion: Collectively, fish oil (omega-3 fatty acids) treatment induced fatty acids catabolism through mediatory role of miR-142-5p and overexpression of ACSL6. Although, the involvement of miR-2368 and transcription factor PPARα is under consideration.
Keywords: Omega-3 Fatty Acids, MicroRNA, Lipid Metabolism, Adipocyte

Ps-64: Construction of Humanized Chimeric Mice through in Utero Transplantation of Human Cord Blood Cells into Mice

Zarrabi M[1, 2], Afzal E[2*], Asghari Vosta Kola Mh[3], Ebrahimi M[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
3. Animal Core Facility, Reproductive Biomedicine Research Center, Royan Institute for Animal Biotechnology, ACECR, Tehran, Iran
Email: mebrahimi@royaninstitute.org

Background: An established alternative to irradiated or genetically immunodeficient mice in transplantation studies is the use of preimmune fetus of animals. We report here the production of humanized chimeric mice by in utero transplantation of human umbilical cord blood cells (UCB-cells) into NMRI mouse fetus. The produced mice can be used as suitable model for study the engraftment and reconstitution potential of human hematopoietic stem cells.
Materials and Methods: On gestational days 11-13, each fetal peritoneum was injected by 3-5×10^6 mononuclear cells or 3-5×10^4 CD34+ cells isolated freshly from UCB. Control animals received only 50µl PBS. The uterine horns were replaced in the abdomen, followed by abdominal closure. Newborn mice were treated with subcutaneous injections of human IL3 (4ng/g), SCF (4ng/g) and GCSF (50ng/g), 3 times a week beginning at 2 weeks of age. To quantitate the level of human cell engraftment, 4 weeks after birth, we performed flow cytometric analysis of peripheral blood from live born mice against human CD45+ marker. Moreover, peripheral blood smears were subjected for human nuclear antigen (HNA) staining.
Results: In utero transplantation of 50 mouse fetuses resulted in 24 live born mice. Human CD45+ cells detected in 63% of infants (range: 1 -5% gated events over controls). More precisely, chimerism was detected in 85% of live born mice whom injected with MNCs, while only 55% of CD34+ transplanted mice had become chimera. Interestingly, the CD45+ cells were detected in both the granulocyte and lymphocyte gates which show multilineage differentiation of engrafted HSCs in transplanted mice.
Conclusion: The identification of circulating human cells in peripheral blood of live born mice supports the engraftment, proliferation and differentiation potential of human cord blood cells in mouse fetuses. Therefore, the presented data show that the technique of in utero HSC transplantation is an appropriate substitute for NOD/SCID/gamma mice in xenotransplantation studies.
Keywords: In UteroTransplantation, Mouse chimera, Cord Blood Hematopoietic Stem Cells

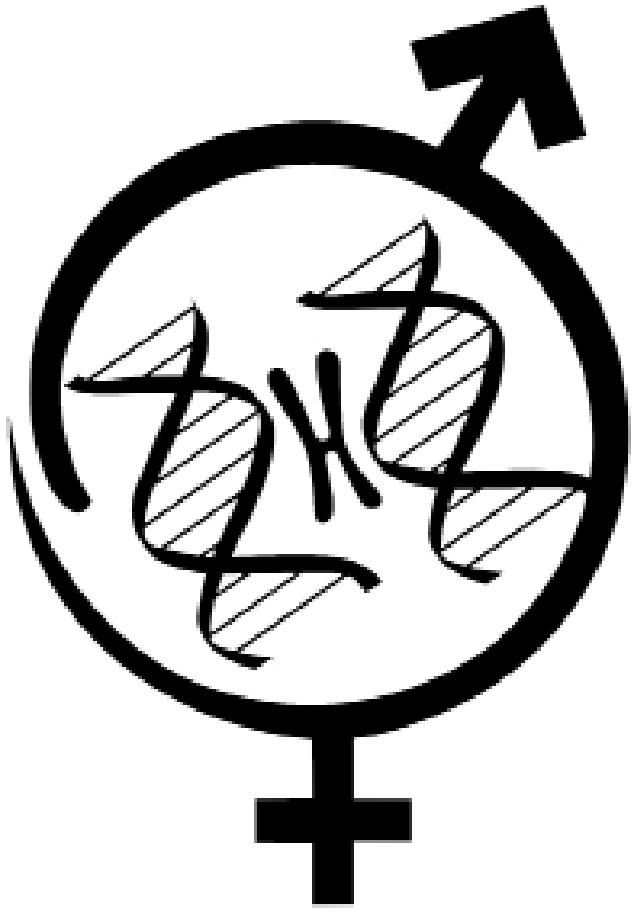
Authors Index

Abstracts of

Royan International Twin Congress

18th Congress on Reproductive Biomedicine
30 August-1 September 2017

12th Seminar on Nursing and Midwifery
30 August-1 September 2017



Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Abstracts of the
18th Congress on Reproductive Biomedicine
12th Seminar on Nursing and Midwifery

Contents

Scientific Board

Collaborators in Award Jury and Scientific Board (Reproductive) 2017

Middle East Fertility Society (MEFS)
Iranian Society for Reproductive Medicine (ISRM), Iran
Iranian Society of Embryology and Reproductive Biology (ISERB), Iran
Iranian Society of Radiology, Iran
Royan Institute, Tehran, Iran
Aalborg University, Denmark
- Department of Health Science and Technology

Afzalipour School of Medicine, Kerman, Iran
- Department of Anatomy

AgResearch Center, New Zealand
- Department of Animal Productivity

AIIMS All India Institute of Medical Sciences, India
- Department of Anatomy

Al-Azhar University, Egypt
- International Islamic Center for Population Studies and Research

Albert Bonniot Research Center Institute, France
- French Institute of Health and Medical Research

Alzahra Hospital, Tabriz, Iran
American Center for Reproductive Medicine, USA
- Cleveland Clinic

Aurigene Discovery Technologies Company, India
Avicenna Research Institute, Tehran, Iran
Babraham Institute, UK
- Epigenetics ISP (Institute Strategic Program)

BioTalentum, Hungary
- BioTalentum Research and Biotalentum Consulting Branch

Brunel University London, UK
- Department of Sociology and Communications

Catholic University of Louvain, Belgium
- Faculty of Medicine and Dentistry

Chinese Academy of Sciences, China
City University of New York, USA
- Department of History, John Jay College of Criminal Justice

Colorado State University, USA
- Department of Biomedical Sciences

Columbia University, USA
- Department of Political Science

Catholic University of Louvain, Belgium
- Department of Gynecology

Erfan Hospital, Infertility Center, Tehran, Iran
Faghihi Hospital, Shiraz, Iran
Federal University of Minas Gerais, Brazil
- Department of Morphology

Florida International University College of Medicine Miami, USA
French National Institute for Agricultural Research, France
- Department of Animal Physiology and Livestock Systems

French National Institute of Health and Medical Research (INSERM)-Grenoble University Research Center, France
Friedrich Loeffler Institute, Germany
- Institute of Farm Animal Genetics

Gazi University, Turkey
- Faculty of Education

Ghent University, Belgium
- Department of Philosophy and Moral Sciences
- Department of Obstetrics, Reproduction and Herd Health

Great Ormond Street Hospital for Children NHS Foundation Trust, UK
- Department of Adolescent Medicine

Guilan University of Medical Science, Iran
- Department of Anatomy, Faculty of Medicine

Hamedan University of Medical Sciences, Iran
- Department of Anatomical Sciences, Medicine Faculty

Harvard University, USA
- Department of Nutrition

Human Reproduction Center, Brazil

Institute for Sterility and Sexuality (ISES), Italy

Institute of Biology and Experimental Medicine, Argentina

Isfahan Infertility Center, Iran

Istanbul University, Turkey
- Institute of Oncology

Italian National Research Council, Italy
- Institute for Organic Syntheses and Photoreactivity ISOF

Karolinska Institute, Sweden
- Department of Women's and Children's Health

Kashan University of Medical Sciences, Iran

KIIT University, India

Kyoto University, Japan
- Laboratory of Reproductive Biology, Graduate School of Agriculture

Laval University, Canada
- Center for Research in Reproductive Biology (CRBR)

Lorestan University of Medical Science, Kamalvand School of Medicine, Iran
- Department of Anatomy

Ludwig-Maximilians-University of Munich, Germany
- Gene Center

Madar Hospital, Yazd, Iran

Mashhad University of Medical Sciences, Iran
- Mashhad IVF Center

Mayo Foundation for Medical Education and Research, USA
- Department of Obstetrics and Gynecology

McGill University, Canada
- Department of Obstetrics and Gynecology
- Division of Urology

Medical Institute of Nasl-e-Omid, Tehran, Iran

Moscow Regional Research Institute of Obstetrics and Gynecology, Moscow, Russia
- Department of Endoscopy

Murdoch Children Institute, Australia

National Institute of Genetic Engineering and Biotechnology (NIGEB), Iran
- Department of Nanobiotechnology

Newlife Fertility Centre in Missisauga, Canada

Ninewells Hospital, USA
- Reproductive and Developmental Biology Group, Centre for Oncology and Molecular Medicine, Division of Medical Sciences

North Carolina State University, USA
- Physiology Graduate Program

Northwestern University, USA
- Department of Obstetrics and Gynecology

Pacific Fertility Center, CA, USA

Pardis Noor Radiology Center, Tehran, Iran

Pasteur Institute, France
- Department of Developmental and Stem Cell Biology

Polytechnic University, Hong Kong
- Department of Applied Social Sciences

Razi University, Kermanshah, Iran
- Department of Biology

Rigshospitalet, Denmark
- Department of Growth and Reproduction

Reproductive Endocrinology and Infertility Mayo Clinic, USA

Royal Victoria Hospital, Canada
- Department of Obstetrics and Gynecology

Shahed University, Tehran, Iran
- Department of Radiology, Mostafa Khomeini Hospital

Saadi Hospital, Shiraz, Iran
- Department of Obstetrics and Gynecology

School of Medicine, Columbia University, USA

Saarlandes University, Germany

Sanger Institute, UK

San Raffaele Scientific Institute, Italy
- Reproductive Sciences Lab, Division of Genetics and Cell Biology

Sapienza University of Rome, Italy
- Department of Anatomical, Histological, Forensic Medicine and Orthopedic Science

Selcuk University, Turkey
- Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine

Shahid Beheshti University of Medical Science, Tehran, Iran
- Department of Biology and Anatomical Sciences, Faculty of Medicine
- Department of Radiology
- Biomedical Ethics Research Center
- Taleghani Hospital, IVF Center
- Cellular and Molecular Research Center
- Mahdieh Hospital

Shahid Chamran University, Ahwaz, Iran
- Department of Statistics, Mathematical Science and Computer Faculty

Shahid Sadoughi Research and Clinical Center, Yazd, Iran

Shiraz IVF and Infertility Center, Iran

Shiraz University of Medical Science, Iran
- Gynecologic Endoscopy Ward
- Department of Obstetrics and Gynecology

Sree Chitra Tirunal Institute for Medical Sciences and Technology, India
- Achutha Menon Centre for Health Science Studies

Superior Institute of Health, Italy

Second University of Naples via Costantinopoli, Italy
- Department of Experimental Medicine

Sheffield Teaching Hospitals NHS Trust, UK
- Centre for Reproductive Medicine and Fertility

Tabriz University of Medical Sciences, Iran

Tarbiat Modares University, Iran
- Faculty of Medical Sciences
- Department of Anatomy

Tehran University of Medical Sciences, Iran
- Department of Infertility, Shariati Hospital
- Department of Epidemiology and Biostatistics, School of Public Health
- Faculty of Theology and Islamic Studies
- Department of Urology, Shariati Hospital

- Department of Medical Ethics, Institute of Endocrinology and Metabolism, Shariati Hospital
- Uro Oncology Research Center, Imam Khomeini Hospital
- Vali-e-Asr Reproductive Health Research Center
- Sina Hospital, Urology Research Center
- Medical Ethics and History of Medicine Research Center
- Department of Radiology; Breast and Women Imaging
- Institute for Environmental Research(IER), Department of Research Methodology and Data Analysis

The Chinese University of Hong Kong, Hong Kong
- Department of Clinical Oncology

The University of Manchester, UK
- Manchester Maternal and Fetal Health Research Centre

University College London, UK
- Institute for Women's Health

University Hospital Coventry and Warwickshire, UK

University Hospital of Basel, Switzerland

University Hospital of Grenoble, France
- Laboratory of Biochemistry and Molecular Genetics Institute of Biology and Pathology

University of Adelaide, Australia
- Robinson Research Institute, Institute for Photonics and Sensing, ARC Centre for Nanoscale Biophotonics

University of Amsterdam LKS, Faculty of Medicine, Netherlands
- Department of Obstetrics and Gynecology

University of Amsterdam (UvA), Netherlands
- Academic Medical Center (AMC)

University of Antwerp, Belgium
- Department of Philosophy

University of Bayreuth, Germany
- Bayreuth Center of Ecology and Environmental Research (BayCEER)

University of California San Francisco, USA

University of Cambridge, UK
- Department of Veterinary Medicine

University of Caxias do Sul (UCS), Brazil
- Institute of Biotechnology

University of Cologne, Germany
- Department of Gynecology and Obstetrics

University of Connecticut, USA
- Center for Regenerative Biology

University of Córdoba, Argentina

University of Florida, USA
- Department of Urology

University of Granada, Spain
- School of Medicine
- Department of Human Anatomy and Embryology

University of Hong Kong, China
- Department of Anatomy, LKS Faculty of Medicine
- Department of Obstetrics and Gynecology

University of Libre Bruxelles, Belgium
- Department of Obstetrics and Gynecology

University of Lowa, USA
- Department of Internal Medicine

University of Leuven, Belgium
- Department of Human Genetics

University of London, UK

University of Melbourne, Australia
- School of Biosciences

University of North Carolina, USA
- Department of Philosophy

University of Oslo, Norway
- Department of Behavioural Sciences in Medicine

University of Otago, New Zealand
- Department of Obstetrics and Gynecology

University of Pavia, Italy

University of Poitiers, France
- Poitiers Research Institute: Research in Materials, Mechanical and Energy Engineering for Transport, Energy and the Environment (Institut Pprime)

University of Porto, Portugal
- Institute for Molecular and Cell Biology

University of Salford, UK
- School of Nursing, Midwifery, Social Work and Social Science

University of Saskatchewan, Canada
- Department of Veterinary Biomedical Sciences

University of Southern California, USA
- Department of Preventive Medicine

University of the Western Cape, South Africa
- Department of Medical Biosciences

University of Toronto, Canada
- Dalla Lana School of Public Health
- Department of Obstetrics and Gynecology

University of Urmia, Iran
- Department of Basic Sciences, Faculty of Veterinary Medicine

University of Ziekenhuis Leuven, Belgium
- Department of Gynecology and Obstetrics

UNSW Sydney, Australia
- Kirby Institute
- School of Women's and Children's Health

USP Dexeus University Institute, Spain
- Department of Obstetrics Gynecology and Reproduction

Victoria University of Wellington, New Zealand
- School of Biological Sciences

Vrije University Amsterdam, Netherlands
- Faculty of Human Movement Sciences

Walter Reed National Military Medical Center, USA
- ART Institute of Washington

Warwick Medical School, UK
- Clinical Sciences Research Institute

Washington State University, USA
- Department of Animal Sciences

Women Hospital, Germany

Yale University, USA
- Department of Obstetrics, Gynecology and Reproductive Sciences

Yazd University of Medical Sciences, Iran
- Yazd Research and Clinical Center for Infertility

Yeshiva University in New York City, USA
- Albert Einstein College of Medicine

Congress Chairperson

Parvaneh Afsharian

Dear Colleagues,

ART centers, around the world, are donating hope and happiness to infertile couples with helping them to have a healthy child. In our area, Royan Institute, as one of pioneers plays a leading role in Reproductive Biomedicine and Stem Cells.

As upcoming Congress Chairperson and on behalf of the Organizing Committee, I would like to welcome each of you to the "18th Royan Reproductive Biomedicine Congress" (RRBC) that will take place from Aug 30 to Sept 1, 2017 in Tehran.

We have devised Scientific Programs with International communities through convention of Annual Royan International Research Award and twin Congress on Reproductive Biomedicine (18th) as well as Stem Cell Biology and Technology (13th).

The Reproductive Biomedicine is an important area in which numerous researches should be conducted to put us on the track of the cutting edge.

The theme of this year congress will be around "Translational Medicine"; it will broadly cover all disciplines of ART protocols and its challenges from fundamental research to "personalized medicine" applications in reproduction highlight global reproductive scientific collaborations.

The organizing committee has intended to provide a wonderful forum for you to meet, interact and exchange your ideas with the Iranian and International outstanding scientists.

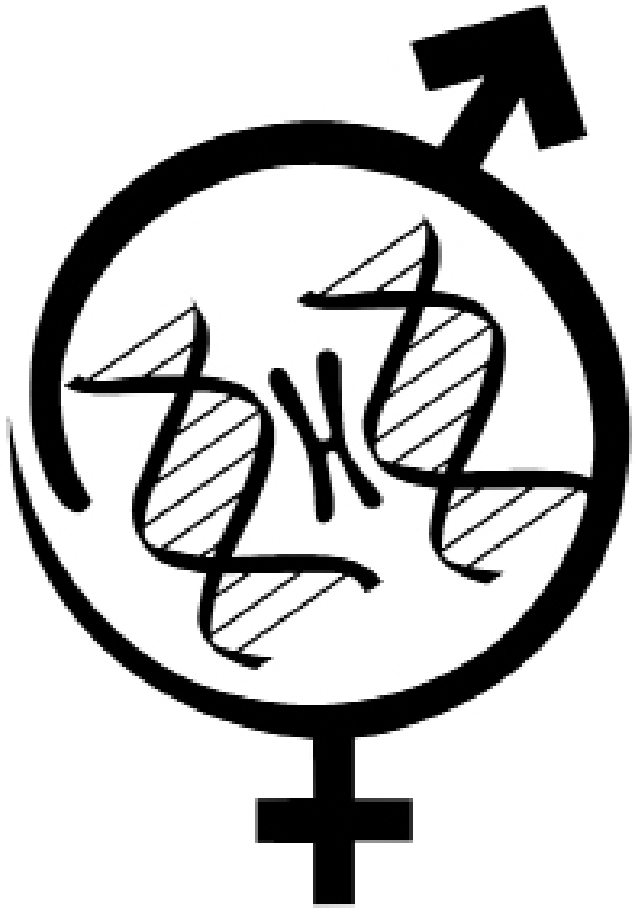
The members of the local organizing committee are very proud to be your host and look forward to welcoming you to our city and country. Furthermore, you will become acquainted with Iran's traditional and modern cultures as the cradle of ancient civilization. I extend personal respect and thanks to all of you.

Parvaneh Afsharian, Ph.D.
Congress Chairperson
18th Royan Congress on Reproductive Biomedicine

Abstracts of

Royan International Twin Congress

18th Congress on Reproductive Biomedicine
30 August-1 September 2017



Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: Male Life Style in ART Outcome

Amirjannati N

Department of Andrology and Embryology, Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: namirjannati@yahoo.com

Lifestyle involves attempt to achieve complete physical, psychological, and social well-being, and includes weight watch, exercise, diet, prevention of diseases, avoiding alcohol and drugs, mental health, spiritual health, social health, increased pregnancy age, and prevention of accidents. Success of ART is largely dependent upon lifestyle. It is entirely possible that changes in lifestyle factors before treatment could lead to a natural restoration of fertility, and could reduce the requirement for ART procedures. Lifestyle factors may affect IVF outcomes, particularly because they are modifiable behaviors that could potentially be altered to enhance the chance of IVF success. Inhaling cigarette smoke can decrease fertility and may affect reproductive outcomes such as causing delayed conception in active male smoking, in addition to active and passive. In men and women consuming four or more alcoholic drinks per week, there was a 48 % decreased chance of fertilization. It is mandatory to consult patients about their lifestyle behavior and to advise them to reconsider their lifestyle in order to improve their IVF chances.

I-2: Untargeted Metabolomic Profiling of Seminal Plasma in Non-Obstructive Azoospermia Men: A Non-Invasive Detection of Spermatogenesis

Gilani K

Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: k.gilany@ari.ir

Background: Male factor infertility affects approximately half of the infertile couples, in spite of many years of research on male infertility treatment and diagnosis; several outstanding questions remain to be addressed. In this regard, metabolomics as a novel field of omics has been suggested to be applied for male infertility problems. A variety of terms associated with metabolite quantity and quality have been established to demonstrate mixtures of metabolites. Despite metabolomics and metabolite analyses have been around more than decades, a limited number of studies concerning male infertility have been carried out. Lack of the ejaculated sperm owing to testicular malfunction has been reported in 6–10% of infertile men, a condition named non-obstructive azoospermia (NOA). In this study, we investigated untargeted metabolomic profiling of the seminal plasma in NOA men using gas chromatography–mass spectrometry (GCMS) and advance chemometrics.
Materials and Methods: Seminal Plasma of fertile men and NOA from positive TESE and negative TESE were collected. The metabolome of were extracted. The metabolome were derivatizaed and analyzed by GCMS. The spectra were process and analyzed by advance chemometrics.
Results: We were able to show based on total ion chromatography (TIC) of different studies groups can be classified by chemometrics analysis. Furthermore, we could identify 36 discriminatory metabolites. These metabolites may be considered discriminatory biomarkers for different groups in NOA.
Conclusion: We have shown in the discovery phase that metabolic profiling can be used to separate the NOA patients. Additionally, we have found 36 potential biomarker to discriminate the different groups in NOA. Furthermore, we have shown metabolic profiling can be use an alternative method to the invasive method TESE to detect spermatogenesis in NOA.
Keywords: Metabolomics, Seminal Plasma, Non-Obstructive Azoospermia, Spermatogenesis, Biomarker

I-3: Sperm DNA Integrity in Cancer Patients before and After Cytotoxic Treatment

Kheradmand A[1, 2]

1. Department of Urology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Infertility Research and Treatment Center of ACECR, Ahvaz, Iran
Email: Kheradmand_a@yahoo.com

DNA is the repository of genetic information in each living cell, its integrity and stability is essential to life. Sperm DNA is six times more compact than in somatic cells, and it is arranged with protamines to form tightly linear side-by-side sheets. Fragmentation or disturbances in DNA arrangement lead to aberrations in sperm function, fertilization, implantation, and pregnancy.
Chemotherapy works by killing cells in the body that are dividing quickly. Since sperm cells divide quickly, they are an easy target for damage by chemotherapy. Permanent infertility can result if all the immature cells in the testicles that divide to make new sperm (spermatogonial stem cells) are damaged to the point that they can no longer produce maturing sperm cells. Despite an early depression in spermatogenesis, a reasonable number of patients show recovery within 1–2 years after treatment with variable sperm counts in their ejaculates.
One concern of both patients and physicians is how much chemotherapy damages the DNA of sperm. Evidence suggests that sperm DNA damage can be detected at least up to 2 years after chemotherapy.
Questions about sperm DNA integrity and mutagenicity after chemotherapy serve as a second reason to encourage men undergoing such oncologic therapy to cryopreserve sperm before induction, because cryopreservation presents a well-established means for fertility preservation in the setting of cancer.

I-4: The Role of Sperm Parameter in ART Outcome

Li HG[1, 2*], Xiong CL[1, 2]

1. Family Planning Research Institute, Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, WUHAN, China
2. Wuhan Tongji Reproductive Medicine Hospital, WUHAN, China
Email: lhgyx@hotmail.com

General sperm parameters, such as sperm concentration or total number of sperm, progressive motility, sperm morphology, sperm acrosome reaction, were found to influence the outcome of assisted reproductive technology (ART), thus have been clinically used to choose the form of ART. However, causes for the ART failure in some couples remain unexplained. Intrinsic parameters related to sperm genetic and epigenetic aspects may account for the ART failure in some patients with male infertility. The correlation between sperm DNA fragmentation and ART outcome have been widely investigated and confirmed. High sperm aneuploidy rate was also found to correlated with ART failure or miscarriage, although there is no good test for sperm aneuploidy rate can be widely applied. However, in men with Klinefelter syndrome (most cases with a 47,XXY karyotype), which is the most common abnormal karyotype in male infertility, the aneuploidy rate of sperm has been shown to be markedly lower than the theoretically calculated. Both sperm retrieval rate and pregnancy rate are close to 50%. Over 100 births from ART have been documented, while only few 47,XXY pregnancies have been documented. Long Y chromosome is usually considered as a normal variant because it is found to not to be related with adverse pregnancy in most studies. Outcomes between intracytoplasmic sperm injection in oligozoospermic men with Y chromosome AZFb or AZFc microdeletions and those of infertile patients with normal Y chromosome showed no significant differences in cleaved embryo rate, high-grade embryo rate, blastocyst formation rate, embryo implantation rate, clinical pregnancy rate and delivery rate. Sperm epimutation and male infertility, transgenerational effect was recently focused. Some studies also have implicated the correlation of sperm epimutation and ART outcome. However, it is difficult to elucidate the correlation and mechanism of sperm epimutation and the ART outcome, due to the sperm epigenetic heterogeneity and inexistence of methods that allow the measurement of epimutation of a single sperm without leading to sperm destruction.

Key words: Sperm, Genetic, Epigenetic, ART Outcome.

I-5: Cell-Free Seminal Nucleic Acids as Novel Noninvasive Biomarkers for Revealing Epigenetic Alterations of Human Testis and Epididymis with Male Infertility

Li HG[1, 2*], Xiong CL[1, 2]

1. Family Planning Research Institute, Center of Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, WUHAN, China

2. Wuhan Tongji Reproductive Medicine Hospital, WUHAN, China

Email: lhgyx@hotmail.com

Background: Previously we have found the presence of cell-free seminal nucleic acids (cfs-NA) in human seminal plasma (Clin Chem 2009, 55:1967-76). And our subsequent researches on the characterization of cfs-NA demonstrated its potential as a noninvasive approach for obtaining the gene expression and epigenetic information of male reproductive organs. Epigenetic aberrations of the testis and epididymis are proposed as the etiology of male infertility because epigenetic regulations are pivotal for sperm production and maturation. However, determination of epigenetic alterations of testis and epididymis in male infertility was hindered by the limitation of obtaining these tissues. On the other hand, a random sampling of the testis may yield an unreliable result because of the heterogeneity of spermatogenesis in testis.

Materials and Methods: By comparing epigenetic profiles of cfs-NA between normozoospermic donors and vasectomized men, whose ejaculates do not contain secretions from the testis and epididymis, we identified the testis and epididymis-specific DNA methylation promoters and miRNAs. To confirm the feasibility of quantification of testicular DNA methylation from cfs-NA, the methylation of some selected testis-specific methylated promoters and the mRNA level of the gene was quantified both in cfs-NA and paired testicular tissues. Methylation of these selected promoters in cfs-NA was then measured in men with nonobstructive azoospermia (NOA) to reveal methylation alterations in NOA patients with different testicular phenotypes.

Results: Promoters of 367 testis and epididymis-specific hypomethylated genes and 134 hypermethylated genes were identified from cfs-NA. Gene Ontology analysis revealed many genes involved in male reproduction. Totally 61 miRNAs in cfs-NA were identified to be predominately derived from the testis and epididymis. We also detected testis-specific circular RNAs and piRNAs in cfs-NA. These epigenetic biomarkers in cfs-NA are stable and unaffected by many clinical factors, such as age, infection, and general storage condition. The feasibility of quantification of testicular/epididymal epigenetic information from cfs-NA was exemplified by promoter methylation. Correlations of methylation of the selected promoters between testicular DNA and paired cfsDNA were observed; and promoter methylation was negatively related to the gene mRNA level in testis. The cfsDNA methylation of these promoters showed different dynamic changes among the subtypes of NOA and normozoospermia. Among them, CCNA1 and DMRT1 promoter methylation in the hypospermatogenesis group was higher than in other groups and showed diagnostic potential for the patient with hypospermatogenesis.

Conclusion: We identified testicular/epididymal epigenetic information, including promoter DNA methylation, miRNAs, piRNAs, and circular RNAs in human cfs-NA. These epigenetic information could be a noninvasive approach for revealing epigenetic alterations of the testis and epididymis in male infertility, which was exemplified by our results of promoter methylation of cfs-NA in NOA patients.

Keywords: Human Testis, Human Epididymis, Epigenetics, Male Infertility, DNA Methylation

I-6: Sperm Retrieval Rate and Reproductive Outcome of Infertile Men with AZFc Deletion

Sadighi Gilani MA[1, 2*], Sabbaghian M[1], Mohseni Meybodi A[3], Zamanian MR[3]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

2. Department of Urology, Shariati Hospital, Tehran University of Medical Sciences,Tehran, Iran.

3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Email: masadighi@gmail.com

Deletions involving the Y chromosome's AZFc region are the most common known genetic cause of severe spermatogenic failure. In the present study we report sperm retrieval rate and reproductive outcome of men with AZFc deletion referred to Royan Institute. AZFc deletion was occurred in 225 patients (58%) amongst 403 cases with AZF deletion. From 195 cases

followed clinical treatments, 116 were azoospermic, 79 were oligozoospermic and most of them (95.8%) had normal karyotype. Of all available pathologies, the predominant trait was SCOS and was seen in 47.6% of cases (49 of 103). Success rate of sperm retrieval in azoospermic patients who underwent MD/TESE was 36.3% (28/77). Hormonal levels were not significantly different in sperm retrieval positive and negative cases. 43 oligozoospermic patients and 17 azoospermic patients started ART cycle. Pregnancy rate in oligozoospermic group was 35.4% (17 cases), while there was no clinical pregnancy in azoospermic group. In conclusion, the pregnancy and delivery in oligozoospermic patients with AZFc deletion are comparable with other studies. On the other hand, despite of sperm retrieval in azoospermic patients with AZFc deletion, the chance of pregnancy or delivery in these patients is very low.

I-7: Male Oncofertility

Sadighi Gilani MA[1, 2]

1. Department of Urology, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: masadighi@gmail.com

The incidence of cancers commonly diagnosed in the adolescent and young adult population, including Hodgkin and non-Hodgkin lymphoma, acute lymphocytic leukemia and testis cancers is on the rise worldwide. Simultaneously, the latest combination chemotherapy regimens have improved the survival rates of these patients to greater than 75-90%, making it possible for many of these young cancer survivors to form a family. Both cancer and cancer treatments can adversely affect a man's ability to father children. Cancer can impair spermatogenesis by hormonal derangements as well as by direct involvement of testicular tissue and the reproductive tract. Additionally, erectile and ejaculatory dysfunction may result from the debilitating physical and emotional impacts of the disease and as side effects of various medical and surgical therapies. The measure of damage as well as the recovery potential is governed by numerous factors. These include the sperm quality before treatment, the type of malignancy and the therapeutic regimen used, e.g., type, dosage and duration of the treatment.9,10. There is no single criterion for predicting the recovery of sperm production. Sperm cryopreservation prior to cancer treatment remains the mainstay of ensuring that a man may father a child in the future with his own sperm. The American Society of Clinical Oncology and the American Society for Reproductive Medicine recommend that, when possible, at-risk patients are referred to a fertility preservation specialist prior to starting cancer treatment.

I-8: Effects of Highly Polluted Environment on Sperm: Telomere Length: A Pilot Study

Vecoli C[1], Montano L[2], Borghini A[1], Notari T[3], Guglielmino A[4], Mercuri A[1], Turchi S[1], Grazia Andreassi M[1*]

1. CNR Institute of Clinical Physiology, Pisa, Italy
2. Andrology Unit of the San Francesco d'Assisi Hospital, ASL Salerno, EcoFoodFertility Project Coordination Unit, Salerno, Italy
3. Infertility Center, ASL Salerno Vallo della Lucania Hospital, Salerno
4. Reproduction Units, HERA Center, Sant'Agata Li Battiati, Catania, Italy
Email: mariagrazia.andreassi@ifc.cnr.it

High environmental pressure may impair male fertility by affecting sperm quality, but the real effect remains controversial. Herein, we assessed the influence of environmental exposure on telomere length (TL) in both leukocytes (LTL) and sperm cells (STL). A pilot biomonitoring study was conducted in 112 clinically healthy, normospermic men living in various areas of Campania region (South of Italy) with high (n = 57, High Group) or low (n = 55, Low Group) environmental pressure. TL analysis was assessed by quantitative real time-PCR. STL was not significantly correlated with either age (p = 0.6) or LTL (p = 0.7), but was significantly longer in the High Group compared with the Low Group (p = 0.04). No significant difference was observed between leukocyte TL in the High or Low Group. Our results showed that male residents in areas with high environment exposure had a significant increase in STL. This finding supports the view that the human semen is a sentinel biomarker of environmental exposure.
Keywords: Environment; Telomere Length; Sperm Quality

I-9: The Role of Human Semen as An Early and Reliable Tool of Environmental Impact Assessment on Human Health in risk areas, Novel Biomarkers of Environmental Pollution: EcoFoodFertility Project

Lorenzetti S[1], Notari T[2], Raimondo S[3], Volpe MG[4], Lannuzzi L[5], Vecoli C[6], Bergamo P[4], Andreassi MG[6], Montano L[7*]

1. Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità (ISS), Rome, Italy
2. Infertility Center, Vallo della Lucania Hospital, Vallo della Lucania, Italy
3. Unit of Seminology, Gentile Research Laboratory, Gragnano, Italy
4. National Research Council, Institute of Food Science, Avellino, Italy
5. National Research Council, Institute for System of Animal Production in the Mediterranean Environment, Naples, Italy
6. National Research Council, Institute of Clinical Physiology, Pisa, Italy
7. Andrology Unit, San Francesco D'Assisi Hospital, Salerno, Italy
Email: l.montano@aslsalerno.it

Background: Reduction of semen quality is reported by several studies. From the "Land of Fires", an area of Southern Italy ill-famed for the multiplicity of sources of pollution (illegal disposal of urban, toxic and industrial wastes, dumping practices, traffic, intensive agriculture) densely populated, who probably has no equal in the world for exposome studies, has been launched a research project named "EcoFoodFertility". This biomonitoring study is expanding to other risk areas of Italy and Europe and utilizes the qualitative and quantitative alterations of human semen, as a key to understand both the level of environmental quality and its long term modifications to set out health risks for populations in relation with their living environment as well as diet and lifestyle. The aims are: i. developing a better understanding of the effects of environmental pollutants on human health in areas with different environmental pressures; ii. qualifying human semen as an early and sensitive environment and health marker; iii. proposing "human seminal model" for early detection and prevention of environmental health risks, useful in innovative programs on health surveillance. and iv. identifying a dietary treatment that can reduce pollutant bioaccumulation and improve human semen quality in

healthy men who live in polluted areas. The present study was conducted on blood and semen of clinically healthy men living in the "Land of Fires" (High Environmental Impact-HEI) and in "Alto-Medio Sele" (Low Environmental Impact–LEI).
Material and Methods: Two different groups of healthy men no smoking, no abitual alcohol drinking, no occupational exposure, were recruited :i) a first group of 110 males(28±5 yrs-old) from HEI (n=60) and LEI(n=50)areas ;ii) a second group of 112 males (29.0±5.6 yrs-old)from HEI(n=57)and LEI(n=55) areas. In the first group,22 trace elements were analyzed in blood and semen by optical emission spectrometry; sperm DNA fragmentation index (DFI)by Sperm Chromatin Dispersion test; total antioxidant capacity (TAC) and antioxidant enzyme activities in the semen (Glutathione reductase, Glutathione peroxidase)by spectrophotometry. In the second group, Telomere Length (TL) was assessed by quantitative Real-Time PCR on genomic DNA extracted from leukocytes(LTL)and sperm(STL).
Results: In the first group, HEI subjects showed significantly higher values($P<0.05$) for Al, Mn, Cr, Mg, Li, Co, Ca in blood, as well as for Cr,Cu,Zn in semen, while Fe was lower in the semen of HEI-group ($P<0.05$).Immotile sperms and the DFI were both higher ($P<0.026$ and $P<0.01$, respectively) in the HEI-group. TAC in blood showed no differences, while TAC, GSR and GpX in the seminal plasma were significantly lower in the HEI-group ($P<0.05$). In the second group, a significant inverse correlation was found between age and LTL ($r=-0.24$, $P=0.01$). STL were not significantly correlated neither to age ($P=0.6$) nor to LTL ($P=0.7$).STL was significantly longer in HEI subjects compared with LEI subjects (0.90 ± 0.26 vs. 1.15 ± 0.51, $P=0.04$). No significant difference was observed between LTL and HEI/LEI areas (0.99 ± 0.33 vs. 1.00 ± 0.38, $P=0.2$). No correlations were seen between STL and sperm parameters.
Conclusion: Semen RedOx status, motility, DFI and STL, can be considered as early markers of environmental pollution and human semen seemed a more early and sensitive source of biomarkers than blood to monitor high environmental pressure on human health, hence useful for innovative prevention programs and health surveillance, especially in risk areas.
Keyword: Semen Quality, Environmental Pollution, Ecofoodfertility, Early Markers, Assessment Human Health

Animal Biotechnology

I-10: ZFP57 Role in The Maintenance of DNA Methylation in Mouse Embryonic Stem Cells

Anvar Z[1, 2, 3, 4*], Riso V[3, 4], Cammisa M[3, 4], Kukreja H[3, 4], Sparago A[3, 4], Riccio A[3, 4]

1. Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Institute of Genetics and Biophysics 'A. Buzzati-Traverso', CNR, 80131 Naples, Italy.
4. Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, 81100 Caserta, Italy,
Email: Zahraanvar2000@yahoo.com

Imprinting Control Regions (ICRs) need to maintain their parental allele-specific DNA methylation during early embryogenesis despite genome-wide demethylation and subsequent de novo methylation. The maintenance of repressive chromatin marks (H3K9me3 and DNA methylation) in this developmental stage is a key regulator of imprinted genes, whose are expressed in a parent-of-origin specific manner.
ZFP57 is a KRAB zinc finger protein. At ICRs the maintenance of methylation is dependent to the assembly of ZFP57 complex on methylated and H3K9me3-bearing allele. Loss of ZFP57 or its co-repressor KAP1 (Trim28) results in loss of DNA methylation and H3K9me3 at ICRs in mouse embryos and embryonic stem cells (ESCs).
By employing genome-wide approaches in mouse ESCs in one of our previous works we identified ZFP57 target sites. Moreover we have shown that *in vitro*, ZFP57 binds a specific hexanucleotide motif (TGCCGC), which is enriched at its target sites. We now demonstrate in mouse ESCs that SNPs disrupting closely-spaced hexanucleotide motifs are associated with lack of ZFP57 binding and H3K9me3 enrichment. We identified a sequence variant (GGCCGC) of the hexanucleotide motif that interacts with ZFP57 both *in vivo* and *in vitro*.
Furthermore to address the role of ZFP57 on all of its target sites we performed high-throughput and multi-locus analyses of inbred and hybrid mouse ESC lines carrying different gene knockouts. We find marked epigenetic differences between ICR and non-ICR targets. ZFP57 is indispensable for the maintenance of methylation at ICRs that in turn is necessary for maintaining the imprinted expression over long distances. At non-ICR targets ZFP57 inactivation results in acquisition of epigenetic features that are characteristic of poised enhancer, suggesting that another function of ZFP57 in early embryogenesis is to repress cis-acting regulatory elements whose activity is not yet required.
Keywords: Embryonic Stem Cells, Imprinted Gene, Methylation Maintenance, ZFP57

I-11: Epigenetics Effects of Cryo-Preservation Methods

Jafarpour F*, Nasr Esfahani MH

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: jafarpour.farnoosh@gmail.com

Assisted reproductive techniques (ARTs) may perturb the pre-/peri-conception microenvironments, which subsequently threaten the health of offspring. One of the most widely used ARTs is vitrification and many concerns were arisen regarding their short- and long-term risks. In our preliminary study, in mouse the effects of superovulation, vitrification, *in vitro* culture, and embryo transfer in terms of expression of epigenetic modulators, imprinted genes, and pluripotency markers in expanded blastocysts and day-9.5 concepti were investigated. These results showed that superovulation, but not vitrification resulted in the most abnormal expression patterns compared to fully *in vivo* developed blastocysts and day 9.5 concepti. It seems that superovulation creates a hormonal environment that negatively affected gene expression and impairs fetal growth more adversely during post-implantation development than other ART protocols. In the next step, we aimed at deciphering the role of vitrification on DNA methylation of H19/IGF2 differentially methylation region (DMR) in *in vitro* produced human blastocysts derived from non-vitrified and vitrified day3 embryos. Methylation level of H19/IGF2 DMR was analysed by bisulfite conversion and sequencing at

18 CpG sites (CpGs) located in this region. Results showed that the overall methylated CpGs percentages of this region in the vitrified and non-vitrified groups were 35.3 ± 3.6% and 38.27 ± 4.1%, respectively (P>0.05). These data may have implications for performing frozen embryo cycles transfer instead of fresh embryo transfer cycles, owing to the naturally synchronized uterus instead of imbalanced hormonal milieu in fresh embryo transfer cycles.

I-12: Amino Acids Turnover in Urea-Exposed Bovine COCs

Kowsar R[1], Norozian Iranshahi V[1], Sadeghi N[2], Ghoreishi SM[3]

1. Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Isfahan, Iran
2. FKA, Animal Husbandry and Agriculture Company, Isfahan, Iran.
3. Department of Animal Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran.
Email: Rasoul_kosarzar@yahoo.com

Dietary protein intake may result in an increase in urea concentration in both blood and follicular fluid. The oocyte quality is drastically influenced by its surrounding immediate microenvironment. Importantly, because of the increased requirement of nutrients for DNA repair processes, uncleaved oocytes have a greater amino acids turnover than cleaving oocytes. The present study reveals that bovine COCs matured in presence of high urea concentration, 40 mg/dl, depleted more amino acids and increased consumption of milk limiting amino acids (methionine and lysine) or key amino acids for oocyte competence (glutamine, alanine, arginine, leucine, and tryptophan). Polynomial basis functions analysis shows various sensitivities (negative to positive) and a biphasic dose response of amino acids to urea. Un-fertilization or cleavage rates also had different sensitivities to amino acids. Taken together, urea changes amino acid turnover and requirements for COCs, disrupting the quiet stage of bovine oocytes. Moreover, the bioinformatics methods can be used to predict the sensitivity of amino acids to urea or the sensitivity of oocyte competence to the different amino acids.

I-13: Application of ART Methods in Saving Endangered Animals

Nasr Esfahani MH

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh_nasr@med.mui.ac.ir

Extinction of species is part of the natural process of evolution, and nowadays this event is occurring at a much higher and faster rate, which is mostly related to human activities such as overhunting. In this regard, modern assisted reproductive technologies (ARTs) including artificial insemination (AI), *in vitro* fertilization (IVF), somatic cell nuclear transfer (SCNT), embryo transfer (ET) and genome resource banking (GRB) are suggested as a potentially integral part of wildlife conservation programs. In practice, these reproductive biotechnologies are species-specific and inefficient for many endangered animals due to limited availability of biomaterials such as sperm and oocytes. Among these wide range of bio-technologies, recent accomplishments in the field of interspecies somatic cell nuclear transfer (iSCNT) hold considerable promise due to its unique potential to decelerate or prevent rapid loss of animal genetic resources, and even to revive extinct species. In this occasion we have tried to explain our successful outcomes of iSCNT technique for conservation of the Esfahan mouflon (Ovis orientalis isphahanica) species in Royan Institute for biotechnology and also explain the challenges which hamper the efficiency of this technique.

Embryology

I-14: Reproductive Tissue Engineering when Bioengineering Meets Reproductive Health

Amorim AC

Faculty of Medicine and Dentistry, Institute for Experimental and Clinical Research (IREC), Pole of Gynecology (GYNE), University Catholique de Louvain, Louvain, Belgium
Email: christiani.amorim@uclouvain.be

I-15: Recent Advances in the Development of a Transplantable Artificial Ovary

Amorim AC

Faculty of Medicine and Dentistry, Institute for Experimental and Clinical Research (IREC), Pole of Gynecology (GYNE), University Catholique de Louvain, Louvain, Belgium
Email: christiani.amorim@uclouvain.be

I-16: Current Sperm Feezing Techniques and Evaluating Protocols in Animals

Bucak MN

Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya/Turkey
Email: mnumanbucak@gmail.com

Objective: The present study was aimed at reviewing of the current freezing and post-thawed evaluation methods of mammalian sperm.
Materials and Methods: Sperm cells are cryopreserved with some upper technological methods as well as conventional systems. These techniques includes liquid nitrogen vapour, dry ice, dried-freezing, encapsulation, directional freezing methods etc. On freezing with these techniques, successful freezing rates is still changeable. In this review, we explain the detail procedures of these techniques, their advantages-disadvantages and studies performed. As evaluating techniques of post-thawed sperm, we make the focus on motility, fluorescent staining (of sperm viability, acrosome and mitochondria integrities), DNA tests (COMET aand TUNNEL) and electron microscopy of sperm abnormalities and insemination techniques.
Results: In this study, we compare the current techniques of sperm freezing, focussing on freezing protocols. As evaluating protocols, parameters providing the more objective results are discussed.
Conclusion: On sperm freezing, some freezing techniques should be improved for increasing the post-thawed sperm parameters and fertility.
Keywords: Sperm Cells, Freezing Techniques, Evaluating Protocols

Is-17: Effects of cryoprotectants and trehalose on ram sperm. An electron microscopic study

Bucak MN[1], Eskin N[2], Çoyan K[2], Pinar I[2], Başpinar N[3], Dursun Ş[4], Güngör Ş[5], Bodu M[1], Acibeava B[1]

1. Selcuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Konya/Turkey
2. Pamukkale University, Faculty of Medicine, Department of Histology and Embryology, Denizli/Turkey
3. Selcuk University, Faculty of Veterinary Medicine, Department of Biochemistry, Konya/Turkey
4. Aksaray University, Faculty of Veterinary Medicine, Department of Gynecology and Obstetrics Gynacology, Aksaray/Turkey
5. Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Burdur/Turkey
Email: mnumanbucak@gmail.com

Objective: The present study was conducted to examine the effects of different cryoprotectants (glycerol, G and ethylene glycol, EG) and trehalose (T) on post-thawed ram sperm morphologies.
Materials and Methods: Ejaculates collected from six Merinos rams, were pooled and evaluated at 37 OC. Pooled semen samples were divided into six aliquots, and diluted in a Tris-based extenders containing 5% G, 3% G+60 mM T, 1.5% G+100 mM T , 5% EG, 3% EG+60 mM T and 1.5% EG+100 mM T. Then, they were cooled to 5 oC and frozen in 0.25 ml French straws. Frozen straws were then thawed individually at 37 oC for 25 s in a water bath for electron microscopy. Field Emission Scanned Electron Microscope (FESEM) was used for examining the thawed sperm. For comparison of the obtained data, the Chi-Square test was used. The differences with values of $p<0.05$ were considered to be statistically significant.
Results: 3% glycerol+60 mM trehalose group provided the highest protection for all sperm morphologies among the groups. All ethylene glycol groups led to higher percentages of undamaged spermatoza, compared to glycerol 5%. The addition of trehalose at different doses in semen extenders tended to reduce the damaged percentages in sperm cells ($P<0.05$).
Conclusion: Trehalose and ethylene glycol supplementation in semen extenders provided a protection of sperm morphologies against cryopreservation injury in electron microscopic examination.
Keywords: Ram semen, cryopreservation, trehalose, cryoprotectant, sperm parameters

I-18: Personalized ART Cycle Management: Individualized Laboratory Technologies

Basile N

Clinical Embryology Laboratory IVI Valencia, Valencia, Spain
Email: nathalia.basile@ivi.es

Since the introduction of the IVF concept in the late 70's the evolution in assisted reproduction has been remarkable. For the time being and until nowadays most of the embryologist's efforts have focused on maximizing the efficiency from oocyte retrieved to the production of highly viable embryos. New technologies have been introduced in the IVF laboratory in order to aid the embryologist to improve outcomes, including those that ascertain embryo has the highest chance of implantation, such as vitrification, co-culture, microfluidics, blastocyst transfer, pre-implantation genetic diagnosis And non-invasive methods of embryo screening as time-lapse, proteomics and metabolomics.
Not all patients follow the same path-flow from oocyte retrieval to embryo transfer. Depending on the number of oocytes recovered or the number of embryos generated, consecutive accumulation following a thawing process may result in an alternative, especially in those cases where mature oocytes are limited or the endometrium is not receptive. On the other hand oocyte and embryo vitrification may also represent a good alternative when the numbers are too high due to an abnormal response and a delay in embryo transfer is necessary.
Once the numbers are satisfactory, strategies extend the days of culture until blastocyst instead of early embryo cleavage stage selection may increase implantation chances. Additionally, time-lapse technology may avoid extended culture, predicting early blastocyst formation or improving culture conditions by providing stable culture conditions and increasing embryo observations (24.7). In this sense, new algorithms for blastocyst prediction and implantation are clinically available and potentially useful for routine practice.
There is room for embryo diagnostic invasive technologies such as CGH arrays being particularly indicated in cases with high risk of aneuploidies such as advanced maternal age or recurrent miscarriage. In these patients, non-invasive technologies would not be able to replace chromosome screening although time-lapse algorithms may be designed to reduce the chances of transferring an abnormal embryo.In the near future embryologist tasks will also change that continuous gamete / embryo handling and assessment will no longer be necessary, improved culture conditions by non-disturbance surveillance and automated procedures will optimize and / or maximize the ratio oocyte retrieved take home baby.

I-19: PGS and Time Lapse in The Detection of Aneuploidies: Versus or Combined?

Basile N

Clinical Embryology Laboratory IVI Valencia, Valencia, Spain
Email: nathalia.basile@ivi.es

The recent introduction of time-lapse systems in the IVF laboratory has solved the problem of applying static and subjective scoring systems in the evaluation of a dynamic process such as embryo development. Semi or completely automatic systems allow uninterrupted surveillance of the embryos and the detection and quantification of various events (e.g. pronuclear formation, syngamy, embryo cleavages, compaction and blastocyst formation). Since that initial step, new kinetic markers associated with higher implantation potential have been proposed, the safety of these systems has been validated, and the effects of different intrinsic and extrinsic factors on kinetic markers has been analyzed.
On the other hand, it is very well known that chromosomal abnormalities are one of the most common causes of abnormal embryos in IVF. The correlation between chromosomal content and embryo morphology and development has been studied based on static observations, however very little is known from a dynamic point of view. A handful of studies have addressed this issue utilizing time-lapse technology to analyze and compare the morphokinetic behavior of chromosomally normal and abnormal embryos. Specific markers related to early and late stages of embryo development have been found to differ between chromosomally normal and abnormal embryos giving rise to algorithms that may increase the probability of selecting chromosomally normal embryos in a non-invasive way.
However, results can be contradictory and some authors suggest that morphokinetic characteristics cannot be used to select

euploid blastocysts in poor-prognosis patients regarded as candidates for pre-implantation genetic screening. Therefore we should be cautious: the selection of embryos through time-lapse technology should not be considered as a replacement to PGS and large multicentre studies are needed in order to clarify the possible relation between morphokinetics and embryo euploidy. However, and specially considering it´s non-invasive nature, there is no harm in utilizing this approach as a selection tool for good prognosis patients that are not indicated for PGS or for patients that are indicated for PGS (history of implantation failure or early pregnancy loss) but that for any legal, social or economic reasons do not wish or can not have PGS performed. In these situations, a clear benefit is gained with morphokinetic screening and selection using a defined algorithm.

I-20: Social Freezing, Where Are We Today?

Basile N

Clinical Embryology Laboratory IVI Valencia, Valencia, Spain
Email: nathalia.basile@ivi.es

Oocyte vitrification is no longer an experimental procedure. In fact, it has become a powerful tool in the management of IVF patients. Who can benefit from it? The answer is diverse: oncologic patients, patients at risk of OHSS, patients with unexpected situations at the day of retrieval and patients electively seeking to preserve their fertility. This last topic is often referred to as "social freezing" and it will be the focus of this lecture.
In the debate about Social Freezing, in society and in the media, the opinion often states that social freezing is primarily used by women who wish to gain more time to pursue their careers. On the contrary, surveys have shown that the overwhelming majority of women who choose social freezing do it because they have not found a partner to start a family. Lack of partner and age are therefore the driving force for these women to cryopreserve their eggs. However it is our duty to inform them about their specific probabilities according to their age at the time of vitrification, making emphasis in the fact that egg freezing does not guarantee success, but increases the possibilities of having a biological child in the future. There is emerging data on the outcomes in this specific group of patients. With respect to age, evidence tells us that there are better chances of having a child when women vitrify their eggs before 35 years old. In contrast to the biological efficiency, the majority of studies show that elective freezing becomes more cost-effective at 37–38 years.
We will first discuss solid data to back-up vitrification as a valid tool to cryopreserve oocytes, then analyze the different types of patients that are electively choosing to cryopreserve their eggs to finnaly discuss outcomes according to the number of eggs vitrified and the age of the patients in order to learn how to advise our patients about their real expectations.

I-21: Dietary Cholesterol-Induced Post-Testicular Infertility

Drevet JR*, Saez F

GReD Laboratory, INSERM U1103/CNRS UMR6293, Université Clermont Auvergne (UCA), Clermont-Ferrand, France
Email: joel.drevet@uca.fr

We bring here the demonstration that an overload of dietary cholesterol causes complete infertility in dyslipidemic male mice (the Liver X Receptor-deficient mouse model). Infertility resulted from post-testicular defects affecting the fertilizing potential of spermatozoa. Spermatozoa of cholesterol-fed lxr-/- animals were found to be dramatically less viable and motile, and highly susceptible to undergo a premature acrosome reaction. We also provide evidence, that this lipid-induced infertility is associated with the accelerated appearance of a highly regionalized epididymal phenotype in segments 1 and 2 of the caput epididymidis that was otherwise only observed in aged LXR-deficient males. The epididymal epithelial phenotype is characterized by peritubular accumulation of cholesteryl ester lipid droplets in smooth muscle cells lining the epididymal duct, leading to their transdifferentiation into foam cells that eventually migrate through the duct wall, a situation that resembles the inflammatory atherosclerotic process. We further show that diet-induced dyslipidemia in LXR-null male mice impairs sperm lipid and protein contents, membrane dynamics, and ability to capacitate. These findings establish the high level of susceptibility of epididymal sperm maturation to dietary cholesterol overload and could partly explain reproductive failures encountered by young dyslipidemic men as well as ageing males wishing to reproduce. When considering that 30% of male infertilities are idiopathic, and that 50% of middle-aged men present an undiagnosed dyslipidemia, a link is possible between male infertility and dyslipidemia.

I-22: Post-testicular Sperm DNA Oxidative Damage: why?where? consequences? from mouse to human

Drevet JR

Genetics Reproduction and Development Laboratory, CNRS UMR62930INSERM U1103-Université Clermont Auvergne, Clermont-Ferrand, France
Email: joel.drevet@uca.fr

Normal embryo and fetal development as well as the health of the progeny are mostly dependent on gamete nuclear integrity. While the oocyte has functional DNA repair mechanisms in place, mature spermatozoa are devoid of them. If sperm DNA damage is too extensive it might surpass the oocyte´s repair capability leading to errors during subsequent DNA replication events after fertilization. To characterize oxidative DNA damage in mammalian sperm we first developed and analyzed mouse models that display high levels of sperm oxidative DNA damage, a common alteration encountered both in in vivo and in vitro reproduction. Immunoprecipitation of oxidized sperm DNA coupled to deep sequencing showed that the vulnerability of chromosomes to oxidative attack inversely correlated with their size. Chromosome position in the sperm nucleus as revealed by fluorescent in situ hybridization is a confounder. Bioinformatic analyses of sperm chromosomal sequences susceptible to oxidation revealed particular features that may help to understand the poor success rates and potential developmental or/and transgenerational risks derived from fertilization events when it involves spermatozoa with oxidative DNA damage. To translate this to the clinic, we then developed an assay allowing a reliable and accurate evaluation of oxidative DNA damage in human sperm. Strong correlations were found with patients bearing abnormal sperm parameters (leucocytospermia and asthenozoospermia) as well as with clinical situations (such as high BMI) known to involved oxidative stress. This assay constitutes a valuable addition to the Andrology/Infertility Clinic laboratory tool box. It allows for a fine evaluation of sperm DNA integrity considered so far as one of the keystone of reproductive success including optimal embryologic development

and low risk of mutation inheritance in the progeny.

I-23: Design of An Optimum Prophylactic Treatment of Oxidative Stress, A Critical Male Infertility Factor

Gharagozloo P

CellOxess Company, LLC, New Jeresey, USA
Email: Parviz.Gharagozloo@CellOxess.com

Background: Oxidative stress is a key driver of adverse alterations of cellular molecular structures such as protein, membrane, RNA and DNA molecules. Spermatogenic cycle or spermatozoa in storage impacted by oxidative stress may have poor motility, low fertilization potential and become senescent or apoptotic. Most importantly, such spermatozoa may carry compromised DNA integrity potentially affecting the normal growth of the embryo and the health of the future generations. Clinical studies confirm moderate to severe sperm oxidative DNA damage in about 60% of all men visiting IVF centers and in about 80% of men diagnosed with idiopathic male infertility. It is therefore of paramount importance that the male partners of couples visiting fertility clinics are tested for oxidative stress or sperm DNA damage and treated correctly with antioxidants prior to performing ART Oxidative stress is a heterogenous pool of many oxidizing metabolites. Although its damaging effects may be partially alleviated by a number of antioxidants or extracts, the strategy for its safe optimum amelioration has remained a challenging scientific endeavor. Over a decade ago, we commenced the design and development of such a prophylactic treatment. Towards this objective, a rigorous examination of the antioxidant literature pertinent to male fertility was conducted. The finding of this work was later published in the July edition of "Human Reproduction" in 2011. A number of natural antioxidants were identified to be essential in combating oxidative stress and the male reproductive health. Each antioxidant ingredient was further evaluated and optimized against a panel of medicinal chemistry criteria such as doses, oral bioavailability, synergy of action with other antioxidants, long term safety, etc. The results of our systematic study led to the invention of a unique formula trademarked Fertilix®. Preclinical studies of this formulation by two independent groups of researchers in France and Spain revealed unequivocal evidence for its efficacy. As a result, this product, classed as "dietary supplement" in USA, has received commercial approval from a number countries including USA, UK, Iran and simultaneously undergoing human clinical evaluations in at least 8 centers in USA and Europe. This presentation will focus on the damaging effects of oxidative stress on male fertility, design of Fertilix formulation together with preclinical data obtained in mouse models of oxidative insult.
Materials and Methods: A comprehensive analysis of the scientific literature was undertaken towards the design of Fertilix formulation. To determine its efficacy, two well-established mouse models of oxidative stress from 2 independent laboratories were employed. In both models, 12 male mice were provided with Fertilix in their drinking water for 2 weeks (Scrotal Heat Shock, SHS) or 4 weeks (Glutathione Peroxidase 5 knock out, GPX-5 KO) and compared with control groups for oxidative DNA damage, epididymal enzymatic antioxidant expression levels, spermatogenic potential, pregnancy rates, fetal resorption and numbers.
Results: The 8-Hydroxy-deoxy Guanosine (8-OHdG) is a biomarker of DNA oxidation. The average background levels of 8-OHdG in WT mice is around 30%. This level doubles up to about 60% in transgenic mice deficient in the antioxidant enzyme GPX-5. Our results indicate that a 2 weeks pretreatment of GPX-5 KO mice with Fertilix provides a complete protection of sperm DNA against oxidation. In mouse models of Scrotal Heat Shock (SHS), only 35% (19/54) female mice got pregnant resulting in 169 fetuses. This is contrast to the Fertilix pretreated group where 74% (42/57) female mice got pregnant resulting in 427 fetuses. The role of chance in obtaining supporting results for the efficacy of Fertilix in both models is minimal.
Conclusion: Fertilix antioxidant formulation is the first scientifically validated formulation, highly efficacious in mouse models oxidative stress impacting sperm cells. It prevents oxidative DNA damage in spermatozoa of GPX-5 deficient mice and restores pregnancy rates almost back to normal levels in mice subjected to SHS. The results obtained in these animal models should have a high likelihood of translating in humans as natural antioxidant molecules have similar functional roles in most mammalian cell types. The results obtained strongly supports the use of antioxidant formulation Fertilix as an adjuvant therapy to a couple's fertility treatment prior to undertaking IUI or IVF procedures.
Keyword: Oxidative Stress, Sperm DNA Damage, Male Infertility, Fertilix, Antioxidants

I-24: Nutrition Counseling in Male Infertility: Dietary Intake or Single Nutraceuticals

Giahi L

Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: dr.lgiahi@gmail.com

Growing market of nutraceuticals are heavily advertised to infertile couples for increasing pregnancy rate worldwide and current literature is full of studies espousing positive effects of nutraceuticals on semen parameters. While, due to the lack of uniformity in design, duration and dosage of studies and absence of control over baseline dietary intake neither scientific literature provide robust evidence, nor the marketed supplements present much proven data on standardization of testing, dosing and careful screening.
Noteworthy, effectiveness of any supplementation in reversing male infertility is highly dependent on lifelong dietary pattern including timing, amount, variety and adequacy of food intake which shapes metabolic responses through epigenetic and neuroendocrine mechanisms from early embryonic stages, infancy, childhood, puberty and adulthood.
Providing individualized dietary solutions which can ameliorate metabolic condition, enrich intake of some nutrients such as omega-3 fatty acids, vitamin E, vitamin C, β-carotene, selenium, zinc, cryptoxantin, lycopene, vitamin D and folate, on one hand, and eliminate the amount of saturated fatty acids and trans-fatty acids and flux of oxidative compounds on the other hand, are needed to be focused prior prescription of any supplement.
Individualized nutrition counseling have to be performed on the basis of comprehensive nutrition assessment, proper understanding of influential factors on prominent dietary habits and realistic consideration of social, cultural, economical and environmental exposures.
However, the importance of preconceptional tailored nutritional counseling and coaching of couples who are trying to conceive

or undergo ART is currently masked by administration of single nutraceuticals which can never reverse or improve infertility in several conditions which mal-dietary pattern, high energy intake and lifelong metabolic adaptations due to dietary habits are important underlying causes of infertility related conditions.

I-25: Spermatogonial Stem Cells: From Bench to Clinic

Koruji M

Cellular and Molecular Research Center and Department of Anatomical Sciences, Iran University of Medical Sciences (IUMS), Tehran, Iran
Email: koruji@iums.ac.ir

The spermatogonial stem cells (SSCs) are unique in mammals because they can transmit genetic information from generation to generation and it is of significant importance. In testes, Sertoli cells, peritubular Myoid cells, Leydig cells and other interstitial cells contribute to the spermatogonial stem cell "niche". So, creation of niche in *in vitro* condition that mimics *in vivo* environment is essential to maintain functional characteristic of SSCs. To this end, several techniques involving SSCs have been developed and will be covered in this presentation. Updates on SSC transplantation techniques with related applications, such as fertility restoration and preservation in cancer patients and culture systems (2D and 3D), are also covered. Since SSC culture holds a pivotal role in SSC biotechnologies, current advances are overviewed.

I-26: Exosomes: A New Paradigm in Embryo-Maternal Cross-Talk for Successful Implantation

Nguyen H[1], Greening D[2*], Simpson R[2], Salamonsen C[1], Eglass K[1]

1. Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia
2. Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science (LIMS)La Trobe University, Melbourne, Australia
Email: D.Greening@latrobe.edu.au

Background: Embryo implantation into receptive endometrium requires synergistic endometrial-blastocyst interactions within the uterine cavity and is essential for establishing pregnancy. Importantly, the point-of-first-contact between the embryo and the maternal-endometrium occurs at the endometrial luminal epithelium. We highlight for the first time a unique insight into the developmental biology of embryo implantation – investigating cellular and secreted changes important for receptivity and implantation, and the contribution of exosomes in regulating this microenvironment.
Materials and Methods: Utilising a combination of cell models, targeted physiologically relevant treatments, quantitative proteomics, and functional real-time assays, we demonstrate endometrial epithelial cellular and secreted protein changes in response to ovarian steroid hormones that drive development of the endometrium to become 'receptive' to an embryo, and to the blastocyst-derived hormone, human chorionic gonadotrophin, which enhances endometrial changes essential for receptivity and implantation. In this study we have defined the proteome of purified endometrial epithelial-derived exosomes (Exos) influenced by menstrual cycle hormones; estrogen (E; proliferative-phase) and estrogen+progesterone (EP; receptive-phase) and examined their potential to modify trophoblast function.
Results: We identified cellular changes associated with metabolism, basement membrane and cell connectivity, proliferation and differentiation, while the secretome analysis identified proteins differentially regulated in associated with cellular adhesion, extracellular-matrix organization, developmental growth, growth factor regulation, and cell signalling. Further, we demonstrate that exosomes (40-150 nm nanovesicles) released from endometrial epithelial cells are an important component of these interactions during receptivity and implantation. Utilizing quantitative proteomics we defined the proteome of purified endometrial epithelial-derived exosomes influenced by menstrual cycle hormones estrogen and progesterone, revealing significant reprogramming associated with cell adhesion, migration, invasion, and extracellular matrix remodeling. In addition to hormonally-treated endometrial cell/secreted and exosomal proteins changes, all findings were validated in human primary uterine epithelial cell-derived material (cells/secretome/exosomes). Functionally, exosomes were internalized by human trophoblast cells and enhanced their adhesive capacity; a response mediated partially through active focal adhesion kinase signaling.
Conclusion: Our results illustrate the dynamic intracellular and secreted protein changes in the endometrium and responses to the pre-implantation embryo, and an active contribution of exosomes to regulating the human uterine environment, that together ensure successful establishment of pregnancy.
Keywords: Embryo Implantation, Exosomes, Pregnancy, Endometrium, Microenvironment

I-27: The Effect of Nanoscale Vibration on *In Vitro* Maturation and Early Embryo Development in Mice

Nikukar H[1, 2*], Khoradmehr A[3], Sadeghian-Nodoushan F[1], Aflatoonian B[1, 2]

1. Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Advanced Biomedical Sciences and Technologies, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: habibnik@ssu.ac.ir

Background: Studies have shown that nanoscale mechanical stimulation named "Nanokicking" could change stem cell fate i.e. increased proliferation rate and induced osteoblastogenesis in endothelial and human mesenchymal stem cell cultures. Also *in vitro* periodic micro vibration has shown an increased implantation rate of human embryo.
We investigated whether nanoscale vibration altered *in vitro* maturation of ovum and embryo development from zygote to blastocyst stage after mice *in vitro* fertilization (IVF).
Materials and Methods: Female mice were superovulated by intraperitoneal injection of pMSG and hCG 48-52 hours later. Mice sacrificed according to National Institute of Health guide for the care and use of laboratory animals and oviducts removed. Ampulla of oviduct was opened and cumulus-oocyte-complexes (COCs) released from within, finally dragged them into drop of GIVF media. Sperm suspension (obtained from cauda epididymidis) added to drop containing COCs. Only two pronucleus (2PN) zygotes, 4-6 hours after insemination

were transferred to G1 media. Nanoscale z-axis vibration was applied by a piezo actuator attached to the Petri dishes' base. Constant sine waves with 20 Vpp amplitude and 1 KHz and 3 KHz frequencies have been tested. After 6, 24, 48, 72, 96 and 120 hours embryo morphology was reported and compared to control.
Results: This method had some effect on *in vitro* maturation of ovum. It also could increase 2PN zygote formation initially after 6 hours compared to others without nanoscale vibration but blastocyst formation decreased afterward. These data need to be supported by more samples and statistical tests.
Conclusion: Mechanical stimulation such as micro and nanoscale vibration could alter maturation of ovum and IVF outcome, but further studies are required to make the mechanism more clear.
Keywords: Mechanobiology, IVM, IVF, Nanoscale Vibration

I-28: Frozen Embryo Transfer: More Success?

Sadeghi MR

Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: sadeghi@avicenna.ac.ir

Repeated implantation failure(RIF) is the failure to perform clinical pregnancy following transfer of at least four top quality embryos at blastocyst stage or at least 10 top quality embryos at cleavage stage in at least three fresh or frozen cycles. It is the main reason for repeated IVF failure. RIF in a significant group of patients is not a stigma or a mysterious pathology, its three main causes are I. Embryo developmental defects (genetics and epigenetics), II. Endometrial developmental defects, (luteal phase deficiency, endometrial receptivity and endometrial thickness), and III. Embryo-endometrial asynchrony(Asynchronous embryo transfer (ET).A large numbers of treatment strategies are proposed for RIF that the impact and effectiveness of them are under debate. One of the impressive treatment options that widely has been used for all of three causes of RIF is the embryo transfer at blastocyst stages in frozen-thawed cycles. Today this policy is going to regarded as routine practice of IVF clinical. In addition PGS using sophisticated molecular methods linked to frozen-thawed cycles help to select the the best euploid embryos that increased success rate up to70- 80%. The most failures of endometrium to implant embryos in RIF patients are not its pathological alteration but rather is a asynchrony of embryo and endometrium. Controlled ovarian stimulation (COH) with pituitary suppression advanced endometrium develoment 2-3 day than natural cycles and so the embryos in fresh cycle are 2-3 later tham endometrium. It was shown that optimal implantation occures when embryo endometrial asynchrony is less than ± 1.5 days. Endometruim advancement in COH could not be attenuated or stop in fresh cycles but we can select the freeze-all policy to overcom this problem. All embryos are frozen in IVF cycles and it followed when single or double embryos are tranferred at blastocyst stage in next natural cycles in synchronization with hormonally primed receptive endometrium with at least adverse effects of exogenouse gonadotropins. A systemic review and meta-analysis on randomized clinical trials only find three reliable RCTs that showed improvement of implantation, clinical, and ongoing pregnancy rates by performing FET compared with fresh embryo transfer. In spite these basic and clinical evidences on supporting FET against fresh ET, but few concerns such as necessity to change current IVF practice, cost increment, more optimization of cryopreservation techniques, epigenetic changes and subsequently large baby of frozen embryo transfer attenuates the replacement of freeze-all policy. By addressing the above concerns and conducting more clinical trials, it is clear that this strategy will be the key point of infertility treatment in future.

I-29: Ovarian Auto-Transplantation in Cancer Patients

Salehnia M

Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: salehnim@modares.ac.ir

Recently the survival and the quality of life in female patients is increasing, however these women are suffering from the side effects of oncological treatments such as endocrine disorders and premature ovarian failures that resulting of infertility. In cancer patients the risk of infertility after chemotherapy or radiotherapy is increased with the age, thus fertility preservation by cryopreservation of oocytes, embryo and ovarian tissues could be proposed.
Autotransplantation of cryopreserved ovarian tissue is doing orthotropic or heterotopic. The first successful report regarding to the human birth from preimplantation of frozen-thawed ovarian tissue was achieved by Donnez group in 2004. After that around 100 babies were born by this method. The pregnancy rate after transplantation of cryopreserved ovarian tissue is reported around 30%. Thus the grafting of cryopreserved ovarian tissue is a useful tool for fertility preservation in cancer patients.

Ethics and Reproductive Health

I-30: Reducing Infertility Burden through Psychology Interventions

Boivin J

Developmental Health and Psychology, School of Psychology, Cardiff University, Cardiff, UK
Email: Boivin@cardiff.ac.uk

Background: To increase knowledge about the sources of psychological and emotional burden in treatment and the psychological techniques that can be used to reduce burden.
Materials and Methods: Review of relevant research.
Results: Infertility comprises the psychological challenges of being childless and the challenges of undergoing treatment. Psychological burden of treatment is significant and can affect quality of life, and [indirectly] pregnancy. The causes of burden can be broadly classified according to whether they originate in the patient, clinic or treatment. Inexpensive psychological techniques can be used to support people coping with fertility problems. Interventions to alleviate these burdens include provision of comprehensive educational material, screening to identify highly distressed patients, provision of tailored coping tools and improvements in the clinic environment and medical interventions.
Conclusion: Compelling longitudinal research demonstrates that patients are better off and more able to reconstruct their lives if they have had a positive experiences trying to resolve their fertility problems. Much has been done to improve the efficacy and safety of fertility treatments. It is now time to turn

attention toward the emotional burden of infertility and its treatment to help patients cope with this prevalent disease of middle adulthood. This challenge needs to be a priority at all levels of healthcare.

I-31: Reducing Infertility Burden through Fertility Awareness And Education

Boivin J

Developmental Health and Psychology, School of Psychology, Cardiff University, Cardiff, UK
Email: Boivin@cardiff.ac.uk

Background: To introduce the topic of fertility awareness and the 'Fertility Status Awareness Tool" (FertiSTAT) which can be used for to educate on the signs, symptoms and preventable causes of fertility problems. The third Sustainable Development Goal is to "Ensure healthy lives and promote well-being for all at all ages". To achieve this target, countries will require reliable, valid and country sensitive methods to increase reproductive knowledge, facilitate access to reproductive health services and support people suffering from reproductive health problems.
Materials and Methods: Review of relevant research
Results: Knowledge about the signs, symptoms and preventable causes of fertility problems is poor in the general population despite a large empirical database about risk factors for fertility problems. The FertiSTAT comprises 22 risk factors for reduced fertility which can be used to help generate a risk profile for reduced fertility, and what can be done to improve the risk profile (timely medical advice, reducing lifestyle factors, monitoring symptoms). The FertiSTAT can achieve high discrimination between medically confirmed fertility and infertile women. A protocol of adaptation for the FertiSTAT has been developed and evaluated in the Middle East using different formats (flipchart, checklist for doctors, self administered).
Conclusions: Healthcare provision for fertility problems must include efforts to prevent fertility problems through education. Inexpensive tools exist to help achieve that goal on a global level.

I-32: Counseling in Assisted Reproductive Failure

Thorn P

The Praxis Center for Couple and Family Therapy, Infertility Counseling, Mörfelden, Germany
Email: mail@pthorn.de

Many patients undergoing ART experience reproductive failure. This includes failure to react adequately to hormonal stimulation, fertilization or implantation failure, early or late loss of pregnancy, ectopic pregnancies, stillbirths, infant death or pregnancy termination for fetal abnormality. Thus, from a psychological perspective, failure is an inherent part of ART and can be an emotional burden both for the individual and the couple. This presentation will explain the emotional experience of failure as well as therapeutic interventions and key components for counselling these patients.

I-33: Counseling of Infertile Couple

Thorn P

The Praxis Center for Couple and Family Therapy, Infertility Counseling, Mörfelden, Germany
Email: mail@pthorn.de

In the last 10 to 15 years, infertility counseling has become valued as an integral part of ART services. In several countries, infertility counseling organizations have been founded and have developed accreditation schemes as well as guidelines for specific aspects of infertility counseling. This presentation will provide an overview of psychosocial care provided by all professionals that have contact to couples undergoing ART and show the specific tasks tackled by infertility counseling. It will give an outline of the qualifications required by counselors and describe some of the future issues that infertility counseling will face.

Female Infertility

I-34: Regenerative Medicine in Reproductive Medicine

Aghdami F

Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: nasser.aghdami@royaninstitute.org

Regenerative medicine has proposed new opportunities for treating patients with reproductive system disorders.
Stem cells are present in embryonic, fetal, and adult tissues and have key roles in tissue repair and homeostasis of the organ and are mentioned as potentially new therapeutic agents in infertility treatment due to their unlimited source and high differentiation potential.
Producing functional gametes from germline progenitors such and iPs are current efforts of scientist in animal studies for infertility management in male and female due to lack of gametes. Stem cell therapy could be another choice in a wider variety of reproductive problems, which do not involve gametes like damaged or thin endometrium or erectile dysfunction.
In Asherman's Syndrome it has been recently proposed that autologous bone marrow derived stem cells transplantation might improve endometrial regeneration in human studies.
In erectile dysfunction there is no clinical trials have been completed but many preclinical studies have been conducted in mouse and rat which indicate that the stem cell transplantation could restore erectile function and penile physiology due to the paracrine factors effect.
In Egypt, studied on potential therapeutic effect of autologous bone marrow mesenchymal stem cells transplantation in women with POF. In ten patients with POF autologous BM-MSC transplanted to their ovaries via laparoscopy. The results revealed recurrence of menstruation in one case after 3 months; two cases showed focal secretory changes in their endometrium after having atrophic endometrium. So, BMSC transplantation seems to have therapeutic effects on POF ovaries.
In ROYAN institute a clinical trial plan has been provided to study on the effects of intra ovarian transplantation of Autologous adipose derived mesenchymal stem cells in POF patients. This phase I study is in enrollment phase.

I-35: Diagnosis and Management of Endometriosis: A Five Year Experience in Iran

Alborzi S

Head of Gynecologic Endoscopy Unit, Shiraz University of Medical Sciences, Shiraz, Iran
Email: alborzisa@gmail.com

Background: The aim of the present study was to review a five year experience on the surgical outcomes of laparoscopic endometriosis surgery.
Materials and Methods: A cohort study was performed in Shiraz University of Medical Sciences using data from medical records of 1315 cases of patients with endometriosis undergoing laparoscopic surgery with follow-up of 6 to 72 months.
Results: This study concerned a cohort of 1315 patients diagnosed with endometriosis operated between April 2011 and April 2016, 1086 (82.5%) of whom were in stage III and IV, 968 (73.61%) had endometrioma (regardless of having DIE or peritoneal involvement), and 347 (26.39%) of patients had either DIE or peritoneal involvement without endometrioma. Regarding endometrioma, left site was more common than right site (355 vs. 291, P=0.002). 646(66.7%) had unilateral endometrioma and 322(33.3%) had bilateral endometrioma.
Regarding deep infiltrative endometriosis which were detected, 825 patients had DIE of uterosacral ligament as the most common site, followed by 344(15.78%) posterior culdesac, 331(15.2%)ovarian fossa, 268 (12.3%) anterior culdesac, 248(11.38%)colorectal, 125 (5.6%) ureter, 33 (1.52%) bladder, 4(0.18%) vagina involvements. Prior to operation, the pain VAS score was 8.23 ± 2.03, which decreased to 4.46 ± 2.47 in 93.07% of patients. Fifty-three patients (6.56%) needed reoperation. Sixty-six (33.1%) of infertile women had spontaneous pregnancy and (25%, n=15) became pregnant using IUI or ART post operatively. Frozen thawed embryo transfer was done for 34 cases out of 51 patients who all had under gone sclerotherapy with no history of laparoscopic intervention. (20.58%) patients of them had clinical pregnancy.
Major complications were occurred in 5 (0.38%), two rectovaginal fistula, two leakage from bowel and one prolong bladder atony, all of them were treated by expectation or second surgery.
Conclusion: Surgical treatment of endometriosis seems to be an effective treatment with very low incidence of major complications. DIE is the most difficult part of operation. Nonetheless, if an expert surgeon performs this procedure, not only the rate of success in symptoms improvement is high, but also the possibility of recurrence of disease would decrease.

I-36: Endometriosis: Basic Sciences Aspects

Amirchaghmaghi E

1. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: amirchaghmaghi_e@yahoo.com

Endometriosis is a common gynecologic disorder and characterized as the implantation of endometrial tissue outside the uterine cavity causing pelvic pain and infertility. Chronic inflammation and sex hormones play important roles in pathophysiology of endometriosis. The elucidation of the molecular pathways mediating growth of endometrial tissues outside the uterus has revealed new insights in diagnosis and treatment of endometriosis. In this talk, we will review new molecular and cellular aspects especially immunologic factors involved in development and regulation of endometriosis.

I-37: Immunologic Aspects of Recurrent Implantation Failure (RIF)

Amirchaghmaghi E

1.Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: amirchaghmaghi_e@yahoo.com

Recurrent implantation failure (RIF) usually refers to failure in achieve a pregnancy after good-quality embryos transfer in a minimum of two or more *in vitro* fertilization-embryo transfer (IVF-ET) cycles. RIF is still a challenging step of ART.
The etiology of RIF mainly includes uterine factors (decreased endometrial receptivity), embryonic defects (embryo factors) and other combined factors. Immunologic factors including immune cells and cytokines network may be one of the most important factors that influence endometrial receptivity and embryo implantation. In this talk, some of important immunologic aspects of RIF will be discussed.
Keywords: Recurrent Implantation Failure, Cytokines, Immune Cells.

I-38: Endometriosis, Life Style, Management

Arefi S

Avicenna Research Institute, ACECR, Tehran, Iran
Email: soheilaarefi@yahoo.com

Exposure to environmental contaminants and hormone disrupting chemicals are associated with a wide spectrum of effects on the reproductive systems. Of particular importance is the potential impact of environmental chemicals on the mucosal immune system of the human female reproductive tract induces endometriosis. Negative effect of endometriosis on folliculogenesis, ovulation, oocyte quality, early embryonic development and implantation in women with endometriosis suggests that infertility in endometriosis patients may be related to alterations within the follicle or oocyte, resulting in embryos with decreased ability to implant. Good life style and regular physical exercise seems to have protective effects against diseases that involve inflammatory processes since it induces an increase in the systemic levels of cytokines with anti-inflammatory properties. In addition, regular physical exercise is associated with a cumulative effect of reduction of menstrual flow, of ovarian stimulation and of the action of estrogen .Also it has been shown that being overweight during late childhood is associated with the development of endometriosis. So it has been proposed that changing life style and regular exercise may influence on progression and recurrence of disease.
Keywords: Endometriosis, Life Style, Exercise

I-39: Assisted Reproductive Outcomes in Women with Different Polycystic Ovary Syndrome Phenotypes: The Predictive Value of Anti-Müllerian Hormone

Ashrafi M[1, 2*], Ramezanali F[1], , Hemat M[2], Arabipour A[2], Jalali S[2], Moini A[2]

1. Department of Obstetrics and Gynaecology, Faculty of Medicine, Iran University of Medical Science, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: ashrafim@royaninstitute.org

Background: The present study was designed to evaluate: 1) ART outcomes in different polycystic ovary syndrome (PCOS) phenotypes compared to control group, 2) the predictive values of serum anti-Müllerian hormone (AMH) and follicle-stimulating hormone (LH/FSH) ratio in PCOS phenotypes for ART outcomes.
Materials and Methods: This cross-sectional study was performed in Royan Institute from June 2012 to January 2014. All infertile women diagnosed with PCOS who underwent the first IVF/ICSI cycle were enrolled during the study period. Other causes of infertility including severe endometriosis, hydrosalpinx, uterine factor, severe male factor (oligo-tetrato-asthenozoospermia), and age factor (≥ 40) or diminished ovarian reserve (AMH<1 ng/ml, FSH>12 IU/l) were excluded. Only patients with mild /moderate male factor and/or tubal factor infertility were included. Meanwhile smokers and diabetic patients were excluded from study. PCOS cases were diagnosed based on the Rotterdam criteria.PCOS patients were categorized to four phenotype groups according to the Rotterdam criteria: phenotype A: the coexistence of hyperandrogenism, chronic anovulation, and polycystic ovaries (HA+AO+PCO); phenotype B: chronic anovulation and hyperandrogenism without the polycystic ovaries (AO+ HA); phenotype C: hyperandrogenism and polycystic ovaries (HA+PCO); and phenotype D: polycystic ovaries coexisting with anovulatory cycles (AO+PCO). In-vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) outcomes in different PCOS phenotypes (A, B, C, and D) compared to control group and the predictive values of serum anti-Müllerian hormone (AMH) in PCOS phenotypes for main outcomes.
Results: In total, 386 cases with a PCOS diagnosis and 350 male factor patients were enrolled during the study period. The women with phenotypes A and C had significantly higher levels of AMH than those with phenotype B. Clinical pregnancy rate (CPR) in the phenotype D group (53.3%) was higher than the other groups (32.5%, 26.4%, and 36.8%, respectively in phenotypes A, B, and C), but it did not reach a significance level. Multivariable regression analysis after adjusting for women`s age and body mass index revealed that PCOS phenotypes A and B were associated with a decreased CPR compared to control group (Odds ratio [OR]: 0.46, confidence interval [CI]: 0.26-0.8, P=0.007 and OR: 0.34, CI: 0.18-0.62, P=0.001, respectively).
Conclusion: It seems that a combination of hyperandrogenism and chronic anovulation is associated with a negative impact on the CPR in these patients. Our results demonstrated that the AMH level is related to PCO morphology but is not predictive for CPR and live birth rate.
Keywords: Polycystic Ovary Syndrome, Phenotypes, Anti-Müllerian Hormone, Assisted Reproductive Technology Outcome, Luteinizing Hormone/Follicle-Stimulating Hormone Ratio

I-40: Minimized Stimulation in ART

Esmaeilzadeh S

Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical sciences, Babol, Iran
Email: sesmael@yahoo.com

In ART, success rates using a spontaneous ovulatory cycle with a single oocyte and a single embryo are low. Various ovarian stimulation strategies have been studied to find a simultaneous development of several oocytes. Hence, the chance of implanting of one of the multiple embryos will enhance at least and as a result, gestational rate increases.
Although, controlled ovarian stimulation (conventional- IVF) increase the number of oocytes obtained, it may diminish endometrial receptivity. It appears that the trend of premature ovarian stimulation increases progesterone and leads to dyssynchrony of endometrium. It interferes with placental development and reduces pregnancy outcome. Probably, embryo quality may have a role as well.
Low-dose stimulation regimens ("light", "soft", "mini", "minimal", "mild", "low cost", or "low dose IVF") are non-conventional controlled ovarian stimulation (non-cCOS). It includes non-controlled (only monitored) ovarian stimulation therapies (non-COS) such as "modified natural IVF" or "antiestrogen/ aromatase inhibitor/low dose FSH-cycles". It decreases the physical, psylological, and financial cost of ART. Besides, it reduces the risk of OHSS and enhances the pregnancy rates at least 10 percent higher.

I-41: Chemotherapy Effects on Ovarian Function in Cancer Patients

Ghaffari F

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: ghafaryf@yahoo.com

Females are born with a fixed number of ovarian primordial follicles. On puberty, approximately 400, 000 follicles remain, which mature in each menstrual cycle. When a healthy woman gets older, follicular depletion accelerates, and the remaining oocytes are of overall poorer quality. The natural follicular depletion presents in an individual female's ovaries can be accelerated by cancer treatment. If the depletion that occurs from cancer treatment is near complete, then the result is acute ovarian failure, i.e., early menopause with consequent infertility that occurs during or shortly after treatment. If the depletion caused by treatment is moderate, then the individual is at risk of premature menopause, i.e., ovarian failure that occurs before the age of 40 years.These patients remain fertile following cancer therapy but have an overall shortened reproductive lifespan. Most chemotherapeutic agents work by affecting cell cycle division. So anticancer agents affect oocytes that are entering the phase of maturation. This mechanism explains the acute impact of chemotherapy on postpubertal women, namely, the cessation of menses during and immediately following therapy. It is less well understood about the impact of chemotherapeutic agents on immature oocytes that have not yet start growing. But, the impact of chemotherapy on reproductive potential depends on the age of the patient at the time of treatment, the particular chemotherapeutic agents used, the duration of treatment, the total cumulative dose administered. acute ovarian failure may be more likely to occur in older women and premature menopause in younger women or adolescents .This is related to the

smaller number of follicles in the ovaries of older patients. Assessing the contribution of individual chemotherapeutic agents on ovarian damage is not easy, It is recommended to describe gonadotoxicity of chemotherapy regimens rather than single agents. Based on studies, we can predict which patients may be at higher risk of gonadotoxicity from their cancer therapy. Older women, women receiving radiation to the ovaries or pelvis, and those receiving regimens that include high-dose alkylating agents represent those at the highest risk for infertility. The patients with highest risk group should be offered fertility preservation prior to cancer therapy.

I-42: Balancing Concept in ARTs: Current Concept via PCOS and IVF: Novel Modalities

Gurgan T

Timur Gurgan Women's Health and IVF Center, Ankara, Turkey
Email: tgurgan@gurganclinic.com.tr

ART has been utilized for the treatment of various fertility problems for almost 40 years with increasing success rates. However, the success rates of the treatment options stabilized for our period of time. There are numerous variables such as the sperms, the oocyte, the embryo, the endometrial receptivity, and the psychosocial status of the couples, which contributes positively or negatively to the success rates.
Recently our team developed a balancing concept on these variables to improve the individual success rates and to personalize the treatment. The concept includes the selection of the sperm by using novel approaches and assures the selection of the most competent sperm cell for fertilisation thus minimizing the possible negative influences of sperm on the embryo. The other arm of the concept is the utilisation of the best possible ovarian stimulation regime in order to have the most viable oocyte. On top of these, the laboratory conditions are kept at the highest standards to facilitate the syngamy of the gametes and for the development of blastocysts for improved implantation potential. The last but not the least arm of the concept is to optimize the receptivity of the endometrium. There are various ways to assess the receptivity of the endometrium using the latest technology. There are also methods to modulate the immunological factors in order to improve the receptivity of the endometrium. Therefore, the aim of this talk is to discuss the various aspects of the balancing concept in a scientific and systematic manner with the possibility of improving your daily practice.

I-43: The Effect of Antioxidants in Female Fertility

Hafezi M

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: maryamhafezi@yahoo.com

Oxidative stress is responsible of pathologic reason of idiopathic infertility, polycystic ovary syndrome and endometriosis. The concentrations of reactive oxygen species (ROS) are low in male and female genital tracts, and high concentration of ROS can cause oxidative stress. ROS can produce from endogenous sources, from different metabolic in human body.
It can be accomplished from exogenous sources such as environmental pollutions (cigarette, alcohol). High concentrations of ROS can cause some pathological effect on cell wall of oocyte and sperm and damage to the DNA of oocyte and spermatozoa.
More over ROS induce implantation failure by altering endometrial receptivity and cause abortion and embryo fragmentation and finally decrease of pregnancy outcome. Antioxidants not only can inhibit ROS and free radicals production but also can repair cell wall damage.
There is some evidence that shows correlation between idiopathic female infertility and high concentration of ROS in peritoneal fluid and some oxidative stress markers such as malondialdehyde (MDA), lipid hydroperoxide (LOOH) and homocysteine (Hcys) are higher in infertile women. Some studies are suggested that plasma and follicular concentration of antioxidant are correlated to higher number of mature oocytes and pregnancy outcome in IVF/ICSI cycles.

I-44: Molecular Detection of Intrauterine Microbial Colonization in Women with Endometriosis

Khan K*, Fujishita A

Department of Obstetrics and Gynecology Kyoto Prefectural University, Center for Quality Research and Development, Kyoto, Japan
Email: nemokhan@koto.kpu-m.ac.jp

Background: Increased intrauterine microbial colonization by bacteria culture method has been reported in women with endometriosis. Here we investigated microbial colonization in intrauterine environment and cystic fluid of women with and without endometriosis by molecular approach.
Materials and Methods: This study was conducted under an approved protocol (No. 26011). With informed consent, a total of 32 women each with and without endometriosis were enrolled. Among them, 16 in each group received treatment with GnRHa. Pattern of microbial colonization in endometrial swabs and endometrioma/non-endometrioma cystic fluid was examined using broad-range polymerase-chain reaction (PCR) amplification of bacteria targeting 16S rRNA gene (rDNA). After quantification of index PCR product, 16S rDNA metagenome sequence analysis was done by Illumina Miseq system.
Results: A wide proportion (0.01-97.8%) of multiple bacteria was detected in both endometrial swabs and cystic fluid. 16S metagenome assay indicated that proportion of Lactobacillacae was significantly decreased ($P<0.01$) and of Streptococcaceae, Staphylococaceae, Enterobacteriaceae was significantly increased ($P<0.05$ for each) in GnRHa-treated women with endometriosis than in GnRHa-untreated women. While bacteria culture method failed to detect a single colony, 16S metagenome assay could detect significantly higher percentage of Streptococcaceae ($P<0.01$) and Staphylococaceae ($P<0.05$) in the cystic fluid derived from women with ovarian endometrioma comparing to that in cystic fluid collected from non-endometrioma cysts.
Conclusion: These findings indicate the occurrence of subclinical infection in intrauterine environment and in the cystic fluid of ovarian endometrioma. Additional side effect of GnRHa treatment in promoting silent intrauterine and/or ovarian infection should be considered. Our current findings may target future therapeutic potential in women with endometriosis and in improving fertility status in these women.
Keywords: Endometriosis, Endometrial Sample, Cystic Fluid, Infection, 16S rDNA Metagenome Assay

I-45: Worldwide ART Practice and Outcome

Mousavifar N

Armaghan Infertility Clinic, Mashhad, Iran
Email: n_m_farr@yahoo.com

Assisted reproduction is increasingly available to infertile and sub-fertile people in many countries. Correspondingly, many different treatment regimens associated with assisted reproduction are being applied to an expanding population of otherwise healthy infertile people.
As a result, the effectiveness, safety, availability and costs of these procedures, as well as the many ethical and legal aspects of their use need specific concern. And also the trend of ART treatment worldwide and its consequences should be precisely monitored.
Most of the reports concerning the ART practice and outcome have come from individual clinical centers and are based on relatively small numbers of patients. Therefore, the applicability of specific results and outcomes from such small and highly selected patients to the general populations seeking treatment is limited.
Therefore, it is crucial to have access to the details of ART treatment globally so that all stakeholders in ART can optimize availability, efficacy, safety, and quality of ART.
This goal can be reached primarily by a national and regional registry. These registries are increasing in number since they enable countries to summarize the total experience of all identified clinics performing assisted reproductive technology treatment. Such registry programs avoid the difficulties in interpreting the results of a small number of clinics performing, for example, limited forms of assisted reproduction on relatively small numbers of patients. Furthermore, an individual clinic can compare its practice and treatment outcomes with others within its country.
The most important an international registry which is important to describe the worldwide use of ART according to availability, effectiveness, safety, and to identify similarities, differences and trends.
In the meeting the latest worldwide result and there responsible organizations will be presented.

I-46: Prenatal Diagnosis

Poransari P

Shahid Beheshti University of Medical Sciences, Shohadaye Tajrish Hospital, Tehran, Iran
Email: pooransarip@yahoo.com

Fetal aneuploidy risk can be evaluated on the basis of a combination of maternal age, prior affected pregnancy or family history, maternal serum biochemical tests and fetal ultrasound markers. New non-invasive prenatal testing based on massively parallel sequencing of circulating free fetal DNA (cfDNA) in maternal plasma has been shown to be highly effective for aneuploidy detection. Potential follow up options for women who are identified as being at high risk based on any of these screening options can include further counseling, additional testing and appropriate follow-up obstetric care. By cell-free fetal DNA (cffDNA) testing, the number of invasive procedures for fetal testing is decreasing dramatically. There are different diagnosing procedures such as CVS, amniosynthesis and chordosynthesis. Amniocentesis should be performed at or beyond 15 + 0 completed weeks of gestation. ChoCVS should not be performed before 10 + 0 completed weeks of gestation, due to the higher risk of fetal loss and complications before this time. CVS should be performed after 10 + 0 gestational weeks. Failure of the cytotrophoblastic culture is reported to occur after fewer than 0.5% of procedures. . Placental cell mosaicism is seen in 1% of procedures.

I-47: New Management of Endometriosis Associated Infertility

Ramezanali F

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: faribaramezanali@royaninstitute.org

Endometriosis is defined as an ectopic presence of endometrial tissue outside the uterus which affects 5-10% 0f women of reproductive age. To approach treatments of different types of endometriosis such as superficial, Deep (DIE), Endometrioma (OMA), we should review the literature and focus especially on articles with a good level of evidence. Endometriosis and infertility are associated clinically. Medical and surgical treatments of endometriosis have different effects on a woman's chances of conception, either spontaneously or via assisted reproductive technologies (ART). Data, mostly uncontrolled, indicate that surgery at any stage of endometriosis enhances the chances of natural conception. Criteria for non-removal of endometriomas are: bilateral cysts, history of past surgery, and altered ovarian reserve. Fears that surgery can alter ovarian function that is already compromised sparked a rule of no surgery before ART. Exceptions to this guidance are pain, hydrosalpinges, and very large endometriomas. Medical treatment -eg. 3-6 months of gonadotropin-releasing hormone analogues-improves the outcome of ART. When age, ovarian reserve, and male and tubal status permit, surgery should be considered immediately so that is dedicated to attempts to conceive naturally. In other cases, the preference is for administration of gonadotropin-releasing hormone analogues before ART, and no surgery beforehand. Weighing up the relative advantages of surgery, medical treatment and ART are the foundations for a global approach to infertility associated with endometriosis.

I-48: Individualized COH on Poor and Hyper Responder

Rashidi B.

Department of Obstetrics and Gynecology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: bhrashidi@yahoo.com

Ovarian Stimulation has been developed over time, but there is no protocol that is ideal for all patients. Hence, the need for Individualization is of great importance.
For Poor responders, there are many therapeutic options; in a recent meta-analysis the clinical outcomes were similar in both GnRH antagonists and GnRH agonists. But in antagonists, the duration of stimulation was less, thereby reducing the burden of treatment.
The physiology of folliculogensis indicates that LH may be necessary in the follicular phase. This hypothesis has been tested in clinical trials with improved efficacy in some subgroups

of women.
Adjuvant therapy with DHEA and Testosterone has not demonstrated consistent support for their use.
In hyper responders, attained evidence from different trials have shown that the measurement of AFC and AMH levels have a greater importance than the female age. These two factors are also being used to predict hyper response which can be resulted to the reduction of OHSS. In women with PCOS, this is a risk category for hyper-responsiveness. After comparing the two protocols, it was concluded that the GnRH antagonist protocol is more efficacious with a lower risk than the GnRH agonist. It has also been stated that, the function of minimal stimulation can be better than conventional regimens which is also associated with lower rates of aneuploidy in the resulting embryos.
The increasing focus on individualization of ovarian stimulation regimens in women undergoing IVF treatment has resulted in screening women who are suspected to ovarian hypo- and hyper-responsiveness with biomarker assessment.

I-49: Role of Uterine and Tubal Disorders in ART Failure

Salehpour S*, Nazari L

Department of Obstetrics and Gynecology, Preventive Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saghar.salehpour2014@gmail.com

Anatomical malformations of the uterus including acquired (fibroids, polyps and adhesions) and congenital (septate and bicornuate uterus) may interfere with implantation and pregnancy.
The receptive endometrium is one of the main factors for implantation and pregnancy. Even in the cases of replacement of chromosomally normal embryos, confirmed by PGS, successful implantation and pregnancy are not reassuring. In a normal menstrual cycle in human, endometrium becomes receptive during the mid-secretory phase around days 19-23 that is described as window of implantation. During this period, cytokines, growth factors, prostaglandins, and adhesion molecules are expressed and inconsistency of these proteins could impair implantation and pregnancy.
Hydrosalpinx may interfere with normal implantation and should be removed to enhance implantation in IVF failure.

I-50: Granulocyte Colony Stimulating Factor in Repeated IVF Failure, A Randomized Trial

Sheikh M[1*], Aleyasin A[2], Abediasl Z[3], Nazari A[2]

1. Maternal, Fetal and Neonatal Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Infertility, Shariati Hospital , Tehran University of Medical Sciences, Tehran, Iran, , Tehran , Iran
3. Infertility Clinic, Bahman Hospital, Tehran, Iran
Email: mahdisheikh@gmail.com

Background: Recent studies have revealed key roles for granulocyte colony stimulating factor (GCSF) in embryo implantation process and maintenance of pregnancy, and some showed promising results by using local intrauterine infusion of GCSF in patients undergoing *in vitro* fertilization (IVF).
Material and Methods: This multi-centric, randomized, controlled trial included 112 infertile women with repeated IVF failure to evaluate the efficacy of systemic single-dose subcutaneous GCSF administration on IVF success in these women. In this study the Long Protocol of ovarian stimulation was used for all participants. Sealed, numbered, envelopes assigned 56 patients to receive subcutaneous 300 µg GCSF before implantation and 56 in the control group. The implantation (number of gestational sacs on the total number of transferred embryos), chemical pregnancy (positive serum β-HCG), and clinical pregnancy (gestational sac and fetal heart) rates were compared between the two groups.
Results: The successful implantation (18% vs. 7.2%, P=0.007), chemical pregnancy (44.6% vs. 19.6%, P=0.005), and clinical pregnancy (37.5% vs. 14.3%, P=0.005) rates were significantly higher in the intervention group than the control group. After adjustment for participants age, endometrial thickness, good quality oocyte counts, number of transferred embryos, and Anti Mullerian Hormone levels, GCSF treatment remained significantly associated with successful implantation (OR=2.63, 95%CI = 1.09 – 6.96), having chemical pregnancy (OR=2.74, 95%CI =1.11 – 7.38), and clinical pregnancy (OR=2.94, 95%CI = 1.23 – 8.33).
Conclusion: Administration of single-dose systemic subcutaneous GCSF before implantation significantly increases the IVF success, implantation and pregnancy rates in infertile women with repeated IVF failure.
Keyword: GCSF, Implantation, Infertility, *In Vitro* Fertilization, Pregnancy

Genetics

I-51: Roles of Aberrantly Expressed Micrornas in Endometriosis

Nasu K*, Narahara H

Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Yufu-shi, Japan
Email: nasu@oita-u.ac.jp

Background: Accumulating evidence suggests that epigenetic aberrations play definite roles in the pathogenesis of endometriosis. MicroRNAs (miRNAs) are a recently defined class of epigenetic mechanism, which is characterized as endogenous, small size, single stranded, non-coding RNA. The purpose of this study is to identify the panel of miRNAs that were aberrantly expressed in primary cultured human endometriotic cyst stromal cells (ECSCs) in comparison with primary cultured normal endometrial stromal cells (NESCs), and evaluate the roles of aberrantly expressed miRNAs in the pathogenesis of endometriosis.
Materials and Methods: ECSCs and NESCs were isolated from ovarian endometriotic tissues and the eutopic endometrial tissues, respectively. Aberrantly expressed miRNAs in ECSCs were identified by a global miRNA microarray analysis. Thereafter, the roles of aberrantly expressed miRNAs regarding the pathogenesis of endometriosis were evaluated by compulsory miRNA expression techniques.
Results: miRNA microarray analysis identified 8 downregulated miRNAs (miR-29b, miR-196b, miR-199a-3p, miR-199b-5p, miR-214, miR-424, miR-455-3p, and miR-503) and 4 upregulated miRNAs (miR-100, miR-132*, miR-181a, and miR-210) in ECSCs. Compulsory expression of miR-196b directed the inhibition of cell proliferation and the induction of apoptosis in ECSCs. miR-503 transfection into ECSCs also induced the cell-cycle arrest at G0/G1 phase and apoptosis, inhibited the cell proliferation, vascular endothelial cell growth factor (VEGF)-A

expression and ECM contractility. miR-196b was found to suppress the mRNA expression of c-myc and B-cell lymphoma/leukemia-2 (Bcl-2) in ECSCs. Cyclin D1, Bcl-2, VEGF-A, Ras homology (Rho) A, and Rho-associated coiled-coil-forming protein kinases were considered as the downstream target molecules of miR-503. Both miR-196b and miR-503 genes were hypermethylated in ECSCs and the treatment with a DNA demethylating agent restored the expression of these miRNAs in ECSCs. The compulsory expression of miR-210 resulted in the induction of cell proliferation, the production of VEGF-A, and the inhibition of apoptosis through signal tranducer and activator of transcription 3 (STAT3) activation in NESCs.
Conclusion: We have identified aberrantly expressed miRNAs which may play important roles in the pathogenesis of endometriosis as a part of epigenetic mechanisms. It is suggested that dysregulated miRNA expressions in ECSCs are involved in the creation of cellular dysfunctions that are disease-specific features of endometriosis.
Keywords: Endometriosis, MicroRNA, Epigenetic, Pathogenesis

I-52: Precision Reproductive Medicine: The Genetic Screening of Preimplantation Embryos

Totonchi M[1, 2], Baba Abbasi B[1], Najafi H[1], Almadani N[1], Eftekhari Yazdi P[3], Rezazadeh M[3], Leigh D[4], Gourabi H[1*]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
3. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Genea, Sydney, NSW 2000, Australia.
Email: gourabi@royaninstitute.org

Background: The concept of personalized medicine builds upon an accurate understanding of the consequences of particular features of individual patients, especially integration of environmental, lifestyle, and genomic variations to improve healthcare. *In vitro* fertilization (IVF) is the most common and effective type of assisted reproductive technology (ART) for treatment of infertility. Although myriad number of studies has investigated the factors affecting pregnancy rates of IVF procedure, preimplatation genetic testing is proposed for screening of embryonic aneuploidy.
Materials and Method: Preimplantation genetic screening (PGS) is applied in patients who have either a history of recurrent abortion or prior ART failures. This study encompasses the data of 124 women who underwent the PGS using array CGH method (2015-2017) at Royan Institute, Tehran Iran.
Results: Initially, the correlations between the relevant factors including: women history of ART failures, history of recurrent abortions, age, the number of oocytes retrieved, the number of embryos transferred, cellular sampling methods, fresh or frozen states and the day of embryo biopsy were assessed with pregnancy rates. Thereafter, by collecting the data of array CGH of 509 embryos, correlation of pregnancy rate with the frequency and types of chromosomal abnormalities was investigated.
Conclusion: In conclusion, by investigations on a population of Iranian infertile women, here we presented a comprehensive study on the most IVF-relevant factors in combination with genetics of embryos, to achieve a more promising IVF procedure.
Keywords: Preimplantation Genetics Screening (PGS), Assisted Reproductive Technology (ART), PGD, Array CGH.

Reproductive Imaging

I-53: Non-Invasive Diagnosis in Endometriosis: New Approaches in Imaging

Haghighatkhah H

Department of Radiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: drhrhaghighatkhah@yahoo.com

Endometriosis is an extra- uterine of endometrial tissue, which lead local inflammatory reaction. More than 176 million women in childbearing age worldwide were estimated to have endometriosis. There is an association between endometriosis and infertility and it is seen in 25-30% infertile woman. Patients with endometriosis commonly have symptoms of heavy menstrual bleeding, congestive dysmenorrhea, fatigue, deep dyspareunia, chronic pelvic pain and dysuria.MRI has provided a simple, feasible and non-invasive tool for diagnosis of clinically suspected patients. Furthermore, it is also useful for both long and short-terms follow up in patients with definite diagnosis of endometriosis. MRI features are atypical due to wide variety of signs and location; however, it can be appeared with hemorrhagic regions with different intensity based on hemoglobin concentration and their age. Recent lesions are usually characterized by hyperintense areas on T1-weighting images, while old lesions are usually hypointense on T1-weighting. Moreover, on T2-weighting images they are appeared with very different intensity but commonly are hypointense. Although MRI has great accuracy in endometriosis identification [8], some cases finally diagnosed with other pathologies. Owing to atypical imaging findings, endometriosis can be misdiagnosed even by expert radiologists. Hemorrhagic cyst, tumoral benign or malignant ovarian cysts and infiltrative pelvic pathologies are the most lesions that can be diagnosed as endometriosis and vice versa. Therefore, laparoscopic or microscopic examination has remained gold standard for the diagnosis of endometriosis. This pictorial assay aims to demonstrate some possible diagnosis of endometriosis on MRI which was inconsistent with histopathological examination.

I-54: Repeated Implantation Failure: New Approaches in Imaging

Malek M

Department of Radiology, Medical Imaging Center, Imam Khomeini Hospital, Tehran University of medical sciences, Tehran, Iran
Email: mmalek@tums.ac.ir

Recurrent implantation failure (RIF) has different causes. The most important causes are uterine abnormalities including, adenomyosis (focal or diffuse), abnormal endometrial cavity (septated, subseptated and arcuate uterus), fibroid , intrauterine abnormalities such as polyp and synechia. To RlO these abnormalities, the first step is a detailed high resolution ultrasound with 3D and sonohysterography for intracavitary and submucosal lesions.
MRI is the last modality to evaluation of junctional zone thickness, position of fibroids, polyp and other intracavitary lesions. hydrosalpynx also could be ruled out by MRI or HSG.

Oral Presentations

Andrology

O-1: Sperm DNA Fragmentation Assays: Evidences, Clinical Usefulness and Implications

Colpi MG, Vaccalluzzo L, Monti V, Testa P, Zaffaroni E

Andrology and IVF Department, Clinica San Carlo, Paderno / Milano (Italy)
Email: gmcolpi@yahoo.com

In the latest years, interest in sperm DNA alterations and their correlations to either spontaneous or ART-induced pregnancy rate has been increasing more and more. Compared to conventional semen analysis, DNA fragmentation (SDF) evaluation could offer more accurate information about male fertility, because its biological variability seems very low (Agarwal, 2016). The available assays are direct or indirect (Evgeni, 2014). The former, by means of probes or dyes, can directly measure SDF (COMET, TUNEL); the latter estimates SDF indirectly, measuring denaturation susceptibility, which is higher in fragmented DNA (SCD, SCSA, Acridine Orange stain [AOS], Aniline Blue staining [ABS]).
Despite all these limitations, however, many studies have pointed out that high SDF levels are associated with a lower pregnancy rate in natural conceptions and after IUI and IVF (Agarwal, 2016), and a higher miscarriage rate after ICSI (Simon, 2017); direct methods may have a higher predictive value of pregnancy outcome (Simon, 2017).
DNA damage may be also due to ROS (Radical Oxigen Species) action. Antioxidant therapy could decrease SDF, but the last metanalysis in literature indicates that this improvement is only ~14% (Cochrane Database, 2014).
SDF levels were found to be eight-times higher in semen samples of varicocele patients compared to controls (Gosalvez, 2015). Surgical correction significantly decreases SDF levels (Zini and Dohle, 2011; Smit, 2013), mainly in II-III degree varicocele (Ni, 2014; Sadek, 2011).
Other studies showed that surgically retrieved sperm have lower SDF rates compared to ejaculated sperm and that their use can improve ART outcomes (Greco, 2005; Esteves, 2015).
These evidences suggest that surgically retrieved sperm could represent a valid therapeutic option in patients showing high SDF levels and with previous failed IVF cycles using ejaculated sperm (Agarwal, 2016; Vu Bach, 2016).
In cryptozoospermia, no evidence has been yet achieved about better ICSI outcomes by using either testicular or ejaculate sperm, although some papers report higher results by surgically retrieved sperm (Weissmann, 2008; Amirjannati, 2012; Popal, 2013; Ketabchy, 2016).
However, in order to provide clinically valid scores, every SDF test requires at least a critical number of sperm cell. TUNEL test seems able to maintain validity even when performed on a low sperm cell number (~200) (Evgeni, 2014); therefore, TUNEL might be useful to determine whether cryptozoospermic patients should perform ICSI with either testicular or ejaculate spermatozoa.
Keywords: DNA fragmentation, semen, ART, male infertility

O-2: Nitric Oxide: Old Friend or Foe in Male Fertility?

Hadwan M*, Almashhedy L[1], Alsalman AR[2]

1. Department of Chemistry, College of Science, University of Babylon, Hilla, Iraq
2. Department of Surgery, College of Medicine, University of Babylon, Hilla, Iraq
Email: mahmoudhadwan@gmail.com

Background: Nitric oxide (NO) is a free radical that is formed by most tissues of the human body and participates in a wide range of biological processes. NO is synthesized through the enzymatic conversion of L-arginine to L-citrulline by the action of one of the isoenzymes known as nitric oxide synthase (NOS), and is concerned with diverse physiological functions in various organs, including the human male reproductive tracts. Several studies have been done to recognize the function of NO in the male reproductive tract after discovery of a unique isoform of NOS as testis-specific nNOS (TnNOS). A comprehensive conclusion from scientific studies recommends that NO in low levels is necessary to complete a group of male reproductive functions such as spermatogenesis, spermiogenesis, sperm motion, acrosome reaction, sperm/oocyte fusion and sperm capacitation. On the other hand, high levels of NO have injurious effects on sperm properties such as motility, morphology and DNA stability. Also, high levels of NO act to increase apoptosis and lipid peroxidation. The current study was described the effect of zinc supplementation on the quantitative and qualitative characteristics of semen, along with enzymes of the NO pathway in the seminal plasma of asthenospermic patients.
Materials and Methods: Semen samples were obtained from 60 fertile and 60 asthenozoospermic infertile men of matched age. The subfertile group was treated with zinc sulfate; each participant took two capsules (220 mg per capsule) per day for 3 months. Semen samples were obtained (before and after zinc sulfate supplementation). After liquefaction of the seminal fluid at room temperature, routine semen analyses were performed. The stable metabolites of NO (nitrite) in seminal plasma were measured by nitrophenol assay. Arginase activity and NO synthase activity were measured spectrophotometrically.
Results: Peroxynitrite levels, arginase activity, NO synthase activity and various sperm parameters were compared among fertile controls and infertile patients (before and after treatment with zinc sulfate). The stable metabolites of nitric oxide (Peroxynitrite) levels and NO synthase activity were significantly higher in the infertile patients compared to the fertile group. Conversely, arginase activity was significantly higher in the fertile group than the infertile patients. Peroxynitrite levels, Arginase activity and NO synthase activity of the infertile patient were restored to normal values after treatment with zinc sulfate. Volume of semen, progressive sperm motility percentage and total normal sperm count were increased after zinc supplementation.
Conclusion: A specific conclusion from the following study suggests that nitric in low levels is necessary to complete a group of male reproductive functions such as spermatogenesis, spermiogenesis, and sperm motion. Treatment of asthenospermic patients with zinc supplementation leads to restored nitric oxide (peroxynitrite) levels, arginase activity and NO synthase activity to normal values and gives a statistically significant improvement of semen parameters compared with controls.

Keywords: Asthenospermia, Nitric Oxide, Nitric oxide Synthase, Arginase, Peroxynitrite

O-3: New Area of Sertoli-Related Niche in Varicocele; Correlation with Spermatogonial Cell Renewal in An Experimental Study

Razi M[1*], Rashtbari H[2], Hassanibafrani H[2], Najaran H[2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran
Email: mazdak.razi@gmail.com

Background: In male gonads, Leydig and Sertoli cells interaction affects spermatogenesis. However, mutual physiologic interaction between these two cells results in acting physiologic Sertoli cells-related niche, which maintains the spermatogonial stem cells (SCCs) renewal, proliferation and differentiation process. Varicocele (VCL), as impairment with abnormal tortuosity of the veins relating to the pampiniform plexus, is known as main male-related infertility disorder. Indeed, crosslink between reduced endocrine potential and failed spermatogenesis in VCLs has been reported. However, the Leydig-Sertoli cells network, Sertoli cells-related niche and the impact of niche on SCCs renewal have been remained unclear. Therefore, present study was performed to uncover the Sertoli-Leydig cells interaction in experimentally-induced VCL by focusing on SCCs renewal machinery.
Materials and Methods: To follow-up current study, 16 mature Wistar rats were divided into control-sham (undergone simple laparotomy) and VCL-induced groups (NO=8 rats in each group). Following two mounts, the Sertoli and Leydig cells distribution/one mm^2 of tissue was analyzed by using SOX-9 and HSD3B immunostainings. Leydig cells steroidogenesis potential was assessed using special fluorescent staining for steroid contents. The Leydig and Sertoli cells were analyzed for mRNA damage using special fluorescent staining. Serum levels of testosterone and inhibin B, as markers reflecting Sertoli and Leydig cells physiologic potential, were analyzed. Finally, expressions of GDNF (representing Sertoli cells-specific element in niche), C-Ret, Etv5 and Bcl-6b (genes involving in SCCs renewal) were evaluated using RT-PCR, immunostaining and Western blot techniques.
Results: Observations showed that, the VCL significantly ($P<0.05$) reduced the Sertoli and Leydig cells number/one mm^2 of tissue versus control-sham group. Moreover, it resulted in intensive mRNA damage and severely reduced serum testosterone and inhibin B levels compared to control-sham animals. The animals in VCL-induced group showed diminished GDNF, C-Ret, Etv5 and Bcl-6 expression in comparison to control-sham group.
Conclusion: The VCL impressively impacts Leydig and Sertoli cells interactions through reducing cells population, steroidogenesis and endocrine status. However, as an update we showed that mentioned evidences adversely affect the expression/synthesis of GDNF as main element initiating renewal process in niche environment. Moreover we showed that, suppressed GDNF expression in line with inhibited C-Ret, Etv5 and Bcl-6 expression/synthesis, results in significant renewal arrest in seminiferous tubules. Thus considering our findings, present study suggests that, Leydig-Sertoli cells network failure in VCLs fairly affects Sertoli-dependent renewal system in niche environment. However, more studies are needed to show alterations in human patients.
Keywords: Varicocele, Leydig Cell, Sertoli Cell, Niche, SCCs Renewal

Embryology

O-4: Sublethal Oxidative Stress to Increase Resistance of Sperm: New Findings for Human Sperm Cryobiology

Hezavehei M[1*], Mohseni Kouchesfahani H[1], Hosseini Salekdeh G[3], Eftekhari-Yazdi P[2], Sharafi M[4], Esmaeeli V[2], Sharbatoghli M[2], Khosravani P[3], Azimi SM[3]

1. Animal Core Facility, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Animal Science, College of Agriculture, Tarbiat Modarres University, Tehran, Iran
Email: Shahverdi@royaninstitute.org

Background: In the present study, different times of mild oxidative stress using NO were considered as novel approach to induce the biosynthesis of resistance related proteins of sperm against cryodamages.
Materials and Methods: Semen samples were collected from forty five normozospermin men to evaluate the primary assessment of ROS-TAC score. Then, twenty four semen samples with same score of ROS-TAC were selected to evaluate with experimental groups. Each Semen samples were washed with PureSperm and then divided into five equal aliquots : fresh group and according to the groups consisting of sperm exposed to NO during the times of 0, 30, 60 and 90 min of pre-freezing incubation. The concentration of NO was applied as 0.01 μM in all of treatments according to previous study. Quality indices sperm were motion characteristics, Apoptotic status and DNA fragmentation using CASA, Caspase activity and SCSA, respectively. Data were analyzed using SPSS and the level of $P<0.05$ was considered as significantly level between compared groups.
Results: There was a significant decrease in all evaluated parameters in the cryopreserved spermatozoa in comparison with the fresh spermatozoa. Furthermore, The higher significant percentage of total, progressive motility, average path velocity and velocity straight linear velocity of frozen-thawed sperm was observed in group with 60 min of pre-freezing sublethaal stress. Moreover, the percentage of caspase 3 activity significantly reduced in this time. the highest significant DNA fragmentation and dead sperm were observed in the time of 90 min. however, sublethal stress in the time of 0 and 30 min before freezing, had not significant difference in case of sperm quality indices.
Conclusion: It seems that oxidative stress in 60 minute before freezing have a crucial role to inhibit the apoptotic pathway resulted from higher expression of stress related protein such as HSPs. We are looking forward to hearing from our proteomics results.
Keywords: Sperm, Cryopreservation, Apoptosis, Sublethal Stress

Genetics

O-5: Creation of Aptamers for Prevention of Fertility Disorders

Kauff A[2], Lin J S[1*]

1. School of Biomedical Sciences, Huaqiao University, Quanzhou, China
2. Reproduction, AgResearch, , Dunedin, New Zealand
Email: junshenglin@hqu.edu.cn

Background: Reproductive activities are driven by the relevant hormones and growth factors. Both follicle-stimulating hormone (FSH) and luteinising hormone (LH) are heterodimers sharing a common structure, α-subunit. The oocyte-derived growth factor bone morphogenetic protein 15 (BMP15) plays important roles in fertility, but its mechanism of action differs between species. Generation of binding molecules to reproductive hormones and growth factors, as an essential investigation tool, would be helpful to provide valuable insight into the underlying biological features of reproductive system at molecule level. The binding molecules could be antibodies or aptamers. Aptamers have many advantages over antibodies as macromolecular ligands for target proteins. Aptamers can be obtained by a method of Systematic Evolution of Ligands by EXponential enrichment (SELEX) beginning with a pool of random sequences. However, the success of this technique cannot be guaranteed if the initial pool lacks candidate sequences. The method of SELEX was modified as a directed *in vitro* evolutionary process. Application of the evolved aptamers were evaluated.
Materials and Methods: Recombinant BMP15 and the common α-subunit of FSH and LH were used as the model targets to generate aptamers. Common PCR reagents plus deoxyinosine triphosphate and $Mncl_2$ were used for mutagenesis PCR. The conventional SELEX was modified as Systematic Evolution of Ligands by Enhanced Mutation (SELEM). The evolutionary process was directed by progressively target-binding selection. Importantly, the selected candidate sequences were subjected to enhanced mutation with the amplification procedure by mutagenesis PCR for the next selection. DNA aptamers could be evolved from a single parent sequence. The evolved aptamers were characterized by Western, eastern, and dot blotting analyses, target-capturing assay, electrophoretic mobility shift assay and measurement of dissociation constant. Functional aptamers were sequenced. A new detective method called Aptamer-based Regionally Protected PCR (ARP-PCR), was developed. The aptamer was allowed to bind to the target protein in solution before digestion with DNase I. The region of the aptamer bound to the target was protected from DNase I cleavage. The target-binding region of the aptamer protected from the enzymatic treatment was then amplified by PCR. The amplified signal reflected only the target-binding region surviving from the enzymatic digestion.
Results: A new method called SELEM has been developed. Aptamers against BMP15 were evolved and identified, which has been used to detect the target in a heterogeneous sample. Aptamers against FSH were evolved and identified. A novel strategy was developed to detect target molecules by ARP-PCR, which is able to detect the target protein at concentrations as low as 10^-14 mol/L.
Conclusion: It was proved that the developed *in vitro* evolution method is able to produce aptamers even the initial pool lacks candidate sequences, which overcomes the intrinsic problem of conventional SELEX. Thereby, functional aptamers against FSH and against BMP15 were successfully created. By combining aptamer technology with the advantages of PCR for amplifying signal, It was proved that the developed ARP-PCR method is a potentially useful analytic tool to detect protein at extreme low concentrations.
Keywords: Aptamer, BMP15, *In Vitro* Evolution, Modified SELEX

O-6: Different Aspects of Toll-Like Receptor 9 Ligation in Trophoblast-Endometrium Interaction

Maslehat N[2*], Sanchez-lopez J[2], Caballero I[2, 3], Spiller D[4], Russell J[5], Elliott S[2], Fazeli A[1, 2]

1. Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
2. Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Sheffield, UK
3. UMR1282 ISP, INRA, Nouzilly, France
4. Oxford Road, The University of Manchester, Manchester, UK
5. The Corporate Information and Computing Services, Sheffield, UK
Email: nasimmaslehat@yahoo.com

Background: Implantation failure caused by sexually transmitted infections (STI) is one of the major factors involved in pregnancy loss. A successful implantation requires a supportive environment, which is strongly dependent on a healthy endometrium. Presence of any infection at the site of the implantation would be sensed by pathogen recognition receptors (PRRs). Toll-like receptors (TLRs) as a major family of PRRs are wildly expressed in the endometrial epithelial cells and are able to react to specific microbial agents, initiate an intracellular signaling pathway, secretion of inflammatory cytokines, which may lead to implantation failure. The aim of the current investigation was to study the TLR9 activation in human endometrial epithelial cells and its effect on the trophoblast behavior in both 2D and 3D *in vitro* cell culture systems.
Materials and Methods: The RL95-2, a Human endometrial epithelial cell line represented the endometrium and the multi-cellular spheroids of JAr cells (a choriocarcinoma cell line) were used to present the embryo, in the 2D culture system. Our 3D culture system was consisted of human endometrial stromal cells (HESC) embedded in a gel (agarose VII) and RL95-2 cells were seeded on top of the gel as a flat monolayer to mimic the endometrium. Finally JAr spheroids were added on top of the system. We evaluated: i. the effect of TLR9 ligation on binding of JAr spheroids to the endometrial cells in the 2D culture system. ii. The TLR9 intracellular signaling pathway in human endometrial epithelial cells in the 2D culture system. iii. The effect of TLR9 ligation on JAr spheroids outgrowth and invasion in the 3D culture system.
Results: Activation of TLR9 in human endometrial epithelial cells had a detrimental effect on binding of trophoblasts to endometrial cells in the 2D culture system. TLR9 intracellular signaling pathway was MyD88 dependent and there were no alteration in activation of NF**k**B and P38 at the time of TLR9 stimulation in endometrial cells. TLR9 activation was able to affect the JAr spheroids outgrowth and invasion in the 3D culture system.
Conclusion: TLR9 activation would trigger an intracellular signaling pathway in endometrium, which is NF**k**B and P38 independent that could affect the outcomes of Human *in vitro*

models of implantation.
Keywords: TLR9, Endometrium, Intracellular Signaling, Pathogen Recognition Receptors (Prrs), Sexually Transmitted Infections (STIS)

O-7: Silymarin and Experimental Endometriosis; Crosslinks Between ERK1/2, Cell Proliferation Apoptosis and Angiogenesis

Nahari E[1*], Razi M[2]

1. Department of Basic Science, Islamic Azad University, Tabriz Branch, Tabriz, Iran
2. Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: mazdak.razi@gmail.com

Background: Endometriosis, phenomenon known as extra-uteri presentation of endometrium, is usually reported with chronic systemic/local inflammatory reactions. Silymarin is known as an antioxidant and anti-inflammatory agent with several therapeutic impacts. As previously shown, the independent pro-inflammatory reactions in ectopic endometrium accelerates cell survival and proliferation in continue of progressive tissue development. Thus, complicated crosslinks between pro/anti-apoptotic proteins and inflammation have been illustrated in legions of patients with endometriosis. However, the role of cell survivors such as B-cell lymphoma 2 (Bcl-2) and Bcl-6b and their cross reaction with extracellular signal-regulated kinases (ERK1/2) and angiogenesis remained unknown. Thus, present study was tried to uncover the antithetical role of ERK1/2 and its relation with oncogenes Bcl-2 and Bcl6b in silymarin (SMN)-treated endometriosis (ENDO)-induced rats.
Materials and Methods: To follow-up current study, experimental ENDO was induced in 14 mature female Wistar rats by autologous transferring of uterine squares (implants) into the intestinal mesentery. Following 21 days, ENDO-induced animals were randomized to two equal groups, including the control (non-treated ENDO-induced) and the SMN-treated (50 mg/kg, orally) groups. Subsequent further 35 days (following 21 days considered for ENDO-induction in rats), the Bcl-2, Bcl-6b and ERK1/2 expressions were appraised by using RT-PCR and immunohistochemical staining (IHC) techniques. Moreover, the apoptosis index as well as angiogenesis ratio were estimated by using TUNEL and special staining for endothelial cells (CD31), respectively.
Results: The RT-PCR analyses exhibited a significant ($P<0.05$) reduction in mRNA levels of Bcl-2 and Bcl-6b in SMN-treated groups compared to non-treated animals. Accordingly, the Bcl-2 and Bcl-6b-positive cells distribution was decreased in SMN-treated group. More analyses showed increased expression of ERK1/2 (both at mRNA and protein levels) as well as enhanced apoptosis index in SMN-treated group. Finally, the animals in SMN-treated group represented diminished angiogenesis ratio versus non-treated animals.
Conclusion: Our data shows that, the SMN by suppressing the Bcl-2 and Bcl-6b expression impressively inhibits cellularity and initiates the intrinsic apoptosis pathway. Moreover, the ERK1/2 as two face element plays a pro-apoptotic role in the absence of Bcl-2, leading to massive DNA fragmentation. On the other hand, the pathologically-produced apoptosis, through mentioned pathways, in turn is able to break the endothelial cells survival/proliferation by degrading the endothelial DNA content. Thereafter, the hypo angiogenesis-related hypoxia will be able to adversely inhibit cellularity and promotes ERK1/2 expression, as well. Taking together, independent and/or dependent to angiogenesis system, the SMN is able to adversely affect the expression of proteins involving in cell survival and/or proliferation.
Keywords: Silymarin, Endometriosis, Cell Proliferation, Apoptosis, Angiogenesis

Reproductive Imaging

O-8: Ultrasonographic Subcutaneous Fat Thickness Measurement and Prediction of Gestational Diabetes Mellitus in The First Trimester

Moukhah S[1*], Ahmadi F[1], Akhbari F[1], Maghari A[2], Hosseini R[2]

1. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. New Hearing Technologies Research Center, Baqiyatallah University of Medical Science, Tehran, Iran
Email: so.moukhah@gmail.com

Background: The aim of this cohort study is to evaluate whether ultrasonographic subcutaneous fat thickness measurement in the early gestational period is useful for predicting the development of gestational diabetes mellitus (GDM).
Materials and Methods: A total of 150 patients were scanned for subcutaneous fat thickness measurement via ultrasound at 11-14 weeks of gestational age for three times. Moreover gestational diabetes mellitus screening was assessed routinely in all patients between 24 to 28 weeks of gestational age as Oral glucose tolerance test(OGTT) that was confirmed by International association of diabetes and pregnancy study groups (IADPSG) in 2013. To obtain, fasting blood sugar (FBS), 1 and 2 hour glucose serum level with 75 gr oral glucose administration was checked in all women. Patients who had one of the values of FBS $\geq$92 mg/dL, 1 h glucose $\geq$180 mg/dL, 2 h glucose $\geq$153 mg/Dl were diagnosed with GDM. Ultrasonograhic subcutaneous fat thickness measurement was compared in women with and without GDM.
Results: GDM was confirmed in 38(25.3%) of patients. There was no significant difference between FBS, 1 and 2 hour glucose serum level among women with and without GDM ($P>0.05$) while subcutaneous fat thickness measurement in two groups was statistically significant ($P=0.035$). The optimal cut-off points for predicting GDM was 20.58 mm [area under curve (AUC) = 0.625, $P=0.23$] for subcutaneous fat thickness measurement.The sensitivity and specificity for this cutoff points were 63.2 and 57.1% respectively.
Conclusion: Ultrasonographic subcutaneous fat thickness measurement in the early period of gestation may be a simply, safe, and cost-effective scan test for predicting the development GDM.
Keywords: Subcutaneous Fat Thickness, Gestational Diabetes Mellitus, Ultrasonography

Poster Speakers

Andrology

P-1: Histochemical Evaluation of Testicular Tissue following The Administration of Methylphenidate and Monosodium Glutamate in Adolescence Rats: Periodic Acid Schiff Reaction

Abdollahzadeh A*, Kianifard D

Department of Basic Sciences, Veterinary Medicine, University of Tabriz, Tabriz, Iran
Email: azadabdollahzadeh@yahoo.com

Background: Methylphenidate is one of the most common medications that used for maintaining alertness and improving of attention which, may lead to increase of the risk of substance abuse in some cases. Monosodium glutamate is a food additive which has toxic effects on human and animal's tissues. Due to the various side effects of methylphenidate and monosodium glutamate on the reproductive system, the aim of this study was to evaluate the effects of these compounds on the alterations of seminiferous tubules basement membrane.
Materials and Methods: Low and high dose of methylphenidate (5 and 10 mg/kg) and monosodium glutamate (6 and 60 mg/kg) was administrated separately and/or in combination form to adolescent rats for 60 days. Testicular tissue samples were stained with periodic acid schiff method and studied under light microscope.
Results: The results showed that, high dose of methylphenidate and low dose of monosodium glutamate and/or combination form of these two compounds have more effects on the increase of basement membrane thickness. Low dose of methylphenidate with high dose of monosodium glutamate influenced the slight alterations of basement membrane. The distinct use of methylphenidate and monosodium glutamate had no significant effect on thickness of basement membrane but, simultaneous use of these compounds led to significant increment of basement membrane thickness.
Conclusion: It has been concluded that, coadministration of methylphenidate and monosodium glutamate can be effective in alteration of thickness of basement membrane of seminiferous tubules and through induction of some changes in glucose transportation could lead to some changes in normal function of reproductive system.
Keywords: Adolescence Rats, Histochemistry, Methylphenidate, Monosodium Glutamate, Periodic Acid Schiff

P-2: Protective Effect of Platelet-Rich-Plasma on Qualitative and Quantitative Parameters of Spermatogenesis in Torsion-Detorsion on Testis in a Mice Model

Aghaei F[1, 2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: aghaei.fatemeh@yahoo.com

Background: Testicular torsion (TT) is a common syndrome that could lead to infertility. In fact, torsion of the spermatic cord is a surgical emergence that results from rotation of the testis and epididymis around the spermatic cord axis. Platelet Rich-Plasma (PRP) may has therapeutic effect due to the presence of growth factors. The aim of this study was investigate the effect of PRP on qualitative and quantitative parameters of spermatogenesis in mice model TT.
Materials and Methods: 18 adult male NMRI mice were randomly and equally divided into three groups: Group 1: Control; TT was not done in this group. Group 2: TT was achieved by twisting the left testis 720 degree for 1 h, then detorsioned. Group 3: Along with TT, mice in this group treated with PRP (10 µl) via local injection in to the left testis after detorsion. 35 days after surgery, left testis was removed and evaluation were made by histomorphometry for qualitative and quantitative parameters study of spermatogenesis.
Results: Mean seminiferous tubular diameter (MSTD), germinal epithelial cell thickness (GECT) and Johnson's score were reduced significantly in TT group in comparison to the control group ($P<0.005$). PRP decreased histopathological score in TT+PRP groups compared to the corresponding TT group.
Conclusion: PRP with its rich growth factor composition has proven beneficial effects in regenerative therapy. PRP is effective for the prevention of testicular torsion damage in mice testis.
Keywords: Torsion, Platelet-Rich-Plasma, Mice, Testis

P-3: Protective Role of GnRH Antagonist from The Intracellular Organelles and Decrease of Apoptosis in Inhibition of Side Effect of Vincristine Used in Chemotherapy on Spermatogenesis

Akbarzadeh A

Department of Histological Sciences, Faculty of Medicine, University of Medical Sciences, Tabriz, Iran
Email: fanik235@gmail.com

Background: There are many factors that creating the cell damage and affect the cell's life, ultimately causing abnormally increased cell death and apoptosis. On the other hand, scientific studies have shown that chemotherapy drugs are the main cause these side effects. Also damaging effects of these drugs is undeniable on intracellular organelles of Reproductive system that is dividing their cells and with the production of cellular damage creates disorders affecting in the reproductive system. The side effect of chemotherapic agents may last from 10 years up to the end of the life since dividing cells are mainly affected by anticancer drugs. The objective of the present review is to investigate the destruction of cellular organelles produced by anticancer drug (Vincristin) and use of the GnRH antagonists is to investigate the protective effect to reduce the side effects of chemotherapy (Vincristin).
Materials and Methods: In the present study 30 adult male mice aging 6-8 weeks were used. The mice were divided into 3 equal groups as: control, Vincristin and GnRH antagonist group. A single dose of vincristine was injected as ip, at 1.5mg/kg. In GnRH antagonist group, cetrorelix injection was started one week before vincristine treatment and continued for 3 more weeks. The mice in all groups were sacrificed 3 weeks after the last dose of Cetrorelix injection. Half of testicular specimens were fixed in 2% glutaraldehyde and prepared for EM studies.

The thin sections were studied with LEO 906 TEM.
Results: Observations electron microscopy in the control group showed that euchromatin nucleus of spermatogonia cells in the form of round and oval which are arranged on basement membrane, Myoid cells are being drawn, Sertoli cells have clear and dense euchromatin nucleus and the shape of a triangle or oval that was the cytoplasm of the basement membrane, Leydig cells were the most important cells in the interstitial tissue that were in a group or individual and had a large nuclear and cytoplasmic euchromatin and multiple Granola with short microvilli, but in the group receiving vincristine that Spermatogenic cells containing organelles are degraded such as Vacuolated of mitochondria and a large number of apoptotic cells with condensed chromatin crescent-shaped and Multi-vesicular bodies (Mb) . Leydig cells were containing more granular than the control group. But In the group receiving GnRH antagonist, Spermatogonia cells similar to control and contain euchromatin nucleus of round and oval, small mitochondria, Endoplasmic reticulum, Golgi and the number of apoptotic cells was reduced. Leydig cells were similar to the control group, but contain numerous granules.
Conclusion: This study showed that the use of GnRH antagonist before and after treatment with vincristine has an inhibitory effect on the Hypothalamic-Hypophysis axis and by reducing in hormones (FSH) and (LH) reduces intracellular organelles damage and apoptosis.
Keywords: GnRH Antagonist, Anticancer Drug, Intracellular Organelles, Apoptosis, Electron Microscopy

P-4: Melatonin Improves Sperm Parameters Along with Serum Levels of Malondialdehyde and Total Antioxidant Capacity in Mice following Treatment with Dexamethasone

Azizi M*, Soleimani Mehranjani M

Department of Biology, University of Arak, Arak, Iran
Email: azizi.m95@yahoo.com

Background: Melatonin as a strong antioxidant hormone which is produced by the pineal gland and has high potential for neutralizing the Pharmaceutical toxins. The aim of this study was to investigate the effect of melatonin on sperm parameters and serum levels of malondialdehyde (MDA) as well as total antioxidant capacity (TAC) in adult mice following treatment with dexamethasone.
Materials and Methods: In this experimental study, male NMRI mice with an average weight of 39 g divided into four groups (n=6) including: control, Dexamethasone (7 mg/kg b.w), Dexamethasone+ Melatonin (7 mg/kg+20 mg/kg) and Melatonin (20 mg/kg). Treatment was performed through intraperitoneally injection for 7days. At the end of treatment period, the blood serum was collected for measuring the level of MDA and total antioxidant potential using thiobarbituric acid method and ferric reducing ability of plasma (FRAP) assay respectively . Then the left caudal epididymis was cut to obtain sperms for evaluating sperm parameters. Data were Statistical analysis using one-way ANOVA and Tukey's test, and the means difference was considered significantly different at $P<0.05$.
Results: A significant reduction in the mean number of sperm was found in the dexamethasone group when compared to the control group ($P<0.001$), this parameter was significantly increased in the dexamethasone+ melatonin group to the control level ($P<0.01$). The mean sperm motility and viability were significantly reduced in the dexamethasone group compared to the control ones ($P<0.001$), while they were increased in the dexamethasone+ melatonin group compared to dexamethasone group ($P<0.001$). A significant increase in the level of serum MDA was seen in the dexamethasone group ($P<0.001$) when compared to the control group, while it was reduced to the control level in the dexamethasone+ melatonin group ($P<0.05$). The mean total of TAC was significantly reduced in the dexamethasone group ($P<0.001$) in comparing with control group, but it was compensated to the control level in the dexamethasone+ melatonin group ($P<0.05$).
Conclusion: Our data showed that the administration of melatonin for 7 days improved motility, count and viability of sperms along with reducing and increasing the serum level of MDA and TAC respectively through reducing oxidative stress in mice treated with dexamethasone.
Keywords: Melatonin, Parameters, Malondialdehyde, Total Antioxidant Capacity, Dexamethasone

P-5: The Protective Effect of Quercetin Against Silver Nanoparticles-induced Testicular Toxicity in NMRI Mice

Bahari HS*, Shariatzadeh SM

Department of Biology, Faculty of Science, University of Arak, Arak, Iran
Email: h.bahary72@yahoo.com

Background: Due to the use of silver nanoparticles in life and production of free radicals and oxidative stress, these particles affect the reproductive system, so the aim of this study was to investigate the effects of quercetin as effective antioxidant on the testes of mice treated with silver nanoparticles.
Materials and Methods: Twenty-four adult male mice(NMRI), were devided into 4 equal groups(n=6) such as : control, silver nanoparticles(500 mg/kg/day), quercetin(50 mg/kg/day) and silver nanoparticles+quercetin.the mouse were treated orally with silver nanoparticles for 35 days and were treated bye intraperitoneal injection with quercetin for 42 days. Finally, the rats were weighted and right testis was removed, fixed, sectioned and stained according to Heiden Hain Azan method. Subsequently the testicular tissue different parameters were studied using stereological methods. Serum testosterone levels were also determined . Data were analyzed by one-way Anova and means difference was considered significant when $P<0.05$.
Results: The total volume of testis, diameter and height of the germinal epithelium, total number spermatids, spermatocytes, sertoli cells significantly decreased in silver nanoparticles group compared to the control group ($P<0.001$). A significant decrease in the serum testosterone levels was found in silver nanoparticles group compared with control group ($P<0.001$). mentioned parameters were largely compensated in silver nanoparticles+quercetin group compared with silver nanoparticles group.
Conclusion: Quercetin seems to could have a protective effect on reproductive system.
Keywords: Silver Nanoparticles, Quercetin, Stereological Methods, Sertoli Cell, Male Mice

P-6: Comparision of The Efficacy of Varicocelectomy in Nonobstructive Azoospermic Men with and without Varicocele

Sepidarkish M[1, 3], Hosseini J[1], Sabbaghian M[1], Sajadi H[1*], Eftekhari yazdi P[1], Dadkhah F[1], Sadighi Gilani M[1, 2], Farrahi F[1]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Urology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Epidemiology Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Email: hesam1979@yahoo.com

Background: To evaluate the outcome of microsurgical varicocelectomy in nonobstructive azoospermic (NOA) men with clinical varicocele in five years in royan institute.
Materials and Methods: A retrospective review of patients treated for NOA and varicocele from march 2011 to march 2016 was performed. Also we have compared MDTESE results of our 57 patients with NOA and clinical varicocele with 537 NOA patients without varicocele in royan institute.
Results: Of 57 patients who underwent varicocelectomy, eight patients (14%) had sperm on sperm analysis postoperatively. One of the patients was single, and one of them had spontaneous pregnancy (1/7)14%, and one had child with microinjection (1/7)14%. Of these 8 patients, 6 had hypospermatogenesis pathology. Of 38 patients who underwent MDTESE , 14 patients (36%) had sperm on testis tissue that one of them had no egg fertilization and so the fertilization rate was (92%). Of these 13 patients, 3 had live child birth (3/13) 23%. Sperm retrieval rate (SRR) in NOA men without clinical varicocele was lower from those who had varicocele and NOA (22 vs. 36%). Also live birth rate in NOA men with varicocelectomy was higher than NOA men without varicocele (23 vs. 11%).
Conclusion: Microsurgical varicocelectomy in NOA men may positive effect on postoperative sperm in ejaculate and spontaneous or assisted pregnancy, but it seems that this effect is more significant on MDTESE results and following successful microinjection. Meanwhile SRR and live birth rate was higher in our patients compare to NOA men without clinical varicocele.
Keywords: Nonobstructive Azoospermia, Varicocele, Sperm Retrieval Rate, MDTESE

P-7: The Influence of Permanent Magnetic Field on Sperm Kinematic Parameters

Ghafelebashi MS[1*], Maghami P[1], Shahverdi AH[2], Dorranian D[3], sabbaghian M[4], Rashki Ghaleno L[2], Sharbatoghli M[2], Esmaeeli V[2], Maroufizadeh S[5]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Department of Physics, Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: m.s_ghafelebashi@yahoo.com

Background: Effect of static magnetic field (SMF) on biological system has been a topic of considerable interest for many years. There is advantage of using permanent magnets for therapeutic applications. Infertility problem is still considered one of the most serious problem of affected couple. This problem exists in all communities one third of infertility endured by couple is due to male factors and among these factor, motility of sperm that one of important parameters in fertility. The objective of this research is to investigate the effects of permanent magnetic field on human spermatozoa motility.
Materials and Methods: After initial examination semen sample were collected from normospermic men (n=30), and were allowed to liquefy for 15-30 min. Each sample was divided into two subsamples that were exposed (‘‘treated’’) or not (‘‘control’’) during, 1-5 h to a uniform static magnetic field at the center of permanent magnetic. During the experiments, a small part of the treated and the control samples was taken away and observed every 60, 180, 300 min from beginning of the treatment. The content of sperm motility was determined by CASA (computer assisted sperm analysis). The assessed motility parameters consisted of: the total motility, (such as (1) the progressive motility; (2) the straight-line velocity (VSL; the straight-line distance from the beginning to the end of a spermatozoa track divided by the elapsed time) given in mm/s and (3) the average path velocity (VAP) given in mm/s. Data analysis was performed using SPSS (version 16) and paired t test. The P value<0.05 is considered significant.
Results: Sperm motility was significantly increased under the influence of static magnetic field while the motility percentage of sham group decreased the motility .The sperm kinematic parameters (VSL, VCL, VAP) were observed increase in the group that was exposed to 1 mt static magnetic field after 1hr exposed.
Conclusion: The static magnetic field could influenced the human sperm motility. The result of this experiment showed the MF at (1mt) after 1hr increased sperm motility .However, sperm velocity was significantly affected by exposure of sperm to MF. The static magnetic field can affect the human sperm motility by increasing the percentage of motile spermatozoa and the correlated kinematic parameters, but these effects depend on both intensity and time of the applied magnetic field.
Keywords: Permanent Magnetic Field , Sperm, Motility

P-8: Mouse Maternal Omega-3 Dietary Fatty Acid with or without Vitamin E Effects on The Offspring’s Sperm Kinematic Characteristics

Ghasemi ZS[1], Ramezani M[1], Alizadeh A[2]

1. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: sadatghasemi@yahoo.com

Background: Vitamin E protects polyunsaturated fatty acid (PUFA) in phospholipids of membrane against peroxidation. We investigated the influence of maternal dietary fish oil (omega-3 source) with and without vitamin E on offspring' sperm parameters.
Materials and Methods: Thirty-six female mice in 6 groups fed different diets adlibitum during one week before mating to weaning day. The dietary groups were standard diet (control;C, 50IU vit.E/kg diet), CLF and CHF groups which consumed 15

and 30 mg fish oil/100g of C diet, respectively. Moreover, a group received vitamin E supplemented diet (E, 125 IU vit.E/kg diet), ELF and EHF groups received 15 and 30 mg fish oil/100g E diet, respectively. All maternal diets contain 3 percent oils which sunflower oil replaced with fish oil. The epididymal spermatozoa were retrieved and sperm parameters were evaluated by CASA (computer- assisted sperm analysis). All data was analyzed by SAS.

Results: Concentration of sperm increased (P<0.05) in vitamin E group. Total motility was higher (P<0.05) in CHF (47 ± 4.22%) and EHF (63 ± 3.56%) than C (41 ± 5.07 %). Progressive motility of sperm affected (P<0.05) by high fish oil added to vitamin E diet (22 ± 3.81% vs. 42 ± 4.69% for C and EHF, respectively). Non-progressive sperms did not differ. The proportion of immotile sperms decreased (P<0.05) when fish oil inclusion in vitamin E diets. Average values of sperm speed; VCL (51 ± 6.91vs. 96 ± 7.25µm/s), VSL (12 ± 2.47vs. 39 ± 4.81µm/s) and VAP (21 ± 3.42 vs. 53 ± 4.45µm/s) (C vs. EHF) were higher when mothers fed high fish oil and vitamin E diet (P<0.05).

Conclusion: Maternal dietary fish oil and vitamin E increased sperm kinematic parameters and they were approximately 2.5 folds greater in combination group (EHF) than others. Therefore, it is necessary to be considered these effects on male offsprings' reproductive when some nutrients recommended during pregnancy and breastfeeding regarding other objectives such as mental and visual function.

Keywords: Maternal Nutrition, Fish Oil, Vitamin E, Sperm

P-9: Varicocele and its Relationship with Hormonal Changes in Infertile Men

Ghasemian F[1*], Zahiri Z[2]

1. Reproductive Health Research Center, Alzahra Educational and Remedial Center, Guilan University of Medical Sciences, Rasht, Iran

2. Department of Biology, University of Guilan, Rasht, Iran

Email: ghasemian@guilan.ac.ir

Background: Varicocele is known a common cause of male factor infertility and its prevalence is approximately 3.58 to 25.4% in the general population. There are many studies about association between varicocele and fertility potential of men. This study evaluates the effects of varicocele on the reproductive hormonal changes among infertile men.

Materials and Methods: A total of 210 infertile men undergoing assisted reproductive treatment were recruited from 2012 to 2015, and assessed the presence of varicocele and hormone levels such as including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. The LH and FSH concentrations of serum were determined with immunofluorometric techniques and Testosterone was measured directly using the Coat-A-Count RIA kit. Binary logistic regression was used to assess the association between varicocele and hormonal changes.

Results: In comparison to men without varicocele and medium hormone levels, there is a significant difference in low and high hormone levels (FSH, LH, and testosterone) for men with varicocele. In other words, the presence of varicocele causes abnormality (increase or decrease) in the levels of FSH, LH, and testosterone hormones (P<0.001).

Conclusion: The increase in FSH and LH hormones were caused by factor such as varicocele, and this factor may influence the fertility potential of men. Future efforts should be made to validate the risk factors for male infertility and strengthen the prevention and treatment of varicocele.

Keywords: Varicocele, Infertile Men, Hormonal Changes

P-10: Effect of Varicocele on Semen Quality and its Prevalence among Infertile Men

Ghasemian F[1*], Zahiri Z[2]

1. Department of Biology, University of Guilan, Rasht, Iran

2. Reproductive Health Research Center, Alzahra Educational and Remedial Center, Guilan University of Medical Sciences, Rasht, Iran

Email: ghasemian@guilan.ac.ir

Background: The most common identified reason of male factor infertility is varicocele. The prevalence of varicocele is found in 15% of the general population. The detrimental effect of varicocele is well known on spermatogenesis, but not completely understood. This study evaluates the effect of unilateral and bilateral varicocele on the semen parameters and its prevalence among different groups of infertile men.

Materials and Methods: A total of 126 infertile men undergoing assisted reproductive treatment were recruited from 2013 to 2014, and assessed the presence of varicocele (unilateral or bilateral) and the semen parameters according to world health organization. The prevalence of varicocele was studied in different groups of male infertility (e.g. asthenozoospermia, oligospermia, teratospermia, azoospermia, normospermia, and oligoasthenoteratozoospermia syndrome). Binary logistic regression was used to assess the association between varicocele and semen parameters.

Results: There is a significant difference in asthenozoospermia rate for men with unilateral varicocele in comparison to other groups of infertile men (P<0.05). This significant difference was also observed in oligoasthenoteratozoospermia syndrome for men with bilateral varicocele in compared with other groups of infertile men (P<0.01).

Conclusion: High rate of asthenozoospermia and oligoasthenoteratozoospermia was observed in infertile men with unilateral and bilateral varicocele, respectively. This result indicated that the presence of varicocele effect on sperm motility and concentration.

Keywords: Varicocele, Infertile Men, Semen Quality

P-11: Peroxiredoxin, An Active Regulator in The Oxidant/ Antioxidant Balance of The Seminal Plasma of Asthenospermic Patients

Hadwan MH

Department of Chemistry, Faculty of Science, University of Babylon, Hilla, Iraq

Email: mahmoudhadwan@gmail.com

Background: Peroxidoxins are thiol (SH) dependent acidic protein having a molecular weight of 20–31 kDa. PRDXs comprise one or two cysteine residues in their active site and are free of heme or selenium. They produce a complex with thioredoxins reductase system to increase their capacity to reduce both organic and inorganic peroxynitrite and hydroperoxides.

Materials 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. Total peroxiredoxin activity; NO synthase activity; arginase activity, glutathione levels; conjugated diene hydroperoxides levels; reactive Oxygen species levels; nitric oxide levels and total antioxidant status were measured spectrophotometrically.
Results: 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, peroxiredoxin activity; arginase activity, glutathione levels; conjugated diene hydroperoxides levels; and total antioxidant were significantly higher in the fertile group than the infertile patients.
Conclusion: Peroxiredoxin activity correlated with antioxidants levels. The decrement of peroxiredoxin activity may be one of the central relations to cause idiopathic asthenospermia.
Keywords: Peroxiredoxin, Arginase, Glutathione, Conjugated Diene Hydroperoxides, Total Antioxidant

P-12: The Effect of Two Types of Aerobic Training on Quality and Quantity Indexes of Sperm in Obese Adult Rats

Hamidian Gh[1*], Raei R[1], Talebi E[2], Saberivand A[3]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
3. Department of Exercise Physiology, Faculty of Sport Science, University of Mazandaran, Babolsar, Iran
Email: hamidian@tabrizu.ac.ir

Background: Overweight and obesity constitute a health problem of increasing prevalence and present a major public health concern. It is a risk factor for non-insulin-dependent diabetes, cardiovascular disease and certain reproductive disorders. It is also associated with disturbance in the hormonal milieu that can affect the reproductive system. On the other hand, lifestyle factors have a dramatic impact on general health and reproductive performance in the general population. The importance of exercise along with a healthy balanced diet is well known to be crucial for health. Therefore, this study was conducted to compare the effect of continuous (CT) and high intensity intermittent aerobic training (HIIT) on male reproductive function in obese adult rats.
Materials and Methods: Thirty adult male wistar rats (Mean ± SE: 176.2 ± 3.73) were homogenously divided into two groups of high fat diet (n: 15) and standard diet regimes (n: 15). After 10 weeks, each group divided into three including: control, CT and HIIT. Training groups completed 10 weeks treadmill running, either HIIT or distance-matched CT on a motorized treadmill. Finally animals were euthanized and their left epididymis was removed and dissected in Ham's F10 solution, incubated at 37°C and sperm motility was evaluated. Total number of sperm per ml was calculated by using a hemocytometer. Smears were prepared from the suspension, stained with eosin-nigrosin and examined for sperm viability and abnormalities by light microscope. Teratozoospermia index (TZI) is defined as the number of abnormalities present per abnormal spermatozoon.
Results: Results showed that in standard diet regimes, CT had no significant effect on sperm number but it could increase all the quality indexes of sperm. It was also indicated that HIIT reduced total number, viability, motility and increased TZI of sperm significantly ($P<0.05$). In obese animals, total number and viability were reduced but these reductions were not significant statistically. Obesity also reduced sperm motility and increased TZI significantly ($P<0.05$) without affecting other sperm parameters. Results revealed that HIIT decreased all quality and quantity index of sperm but CT improved side effect of obesity on these indexes as to reach to normal levels.
Conclusion: It can be concluded that obesity can affect sperm quality and caused a reduction in fertility potent. However HIIT reduced quality and quantity parameters of sperm but CT can improve these parameters in normal and obese animals and have a protective and supportive effect on impairment caused by obesity in male fertility.
Keywords: Aerobic Training, Sperm Analysis, HIIT, CT, Obesity

P-13: The Effect of Methanolic Extract of Coconut Meat on Quality and Quantity Indexes of Sperm in Type II Diabetic Rats

Hamidian Gh[1*], Asghari K[1], Raei P[1], Hasanzadeh M[1], Hazaveh M[1], Ozeiry P[1], Mahmoudi R[2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
Email: hamidian@tabrizu.ac.ir

Background: Diabetes mellitus (DM) is one of the greatest public health threats in modern societies. There is a strong association between male infertility and DM. The increasing incidence of DM worldwide will inevitably result in a higher prevalence of this pathology in men of reproductive age and subfertility or infertility associated with DM is expected to dramatically rise in upcoming years. Using new potent therapeutic regimens containing natural materials in the treatment of metabolic complications can be interesting because of their multipotent potentials, safety and lower adverse and side effects. Coconut is one of the highest nutritional and medicinal value plants with various fractions which play a major role in several biological applications but there is no report about its effects oil on male reproductive function in diabetic peoples.
Materials and Methods: In this study 25 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 methanolic extract of coconut meat (MECM) by oral gavage for 40 consecutive days. Type II diabetes was induced by high fat diet and 35 mg/kg streptozotocin in diabetic and treatment groups. In the end of the experimental period, animals were euthanized and their left epididymis was removed and dissected in Ham's F10 solution, incubated at 37°C and sperm motility was evaluated. Total number of sperm per ml was calculated by using a hemocytometer. Smears were prepared from the suspension, stained with eosin-nigrosin and examined for sperm viability and abnormalities by light microscope.
Results: Results showed that diabetes reduced all of sperm parameters significantly compared to control group ($P<0.05$). It was indicated that MECM could increase quality and quantity indexes of sperm in a dose dependent manner significantly. However increasing in total number of sperm by low and mid dose of MECM was not significant compared to diabetic

group but motility and viability of sperm were increased in both doses. Results also revealed that administration of high dose of MECM had the best effect on sperm parameter of diabetic rats.
Conclusion: It can be concluded that coconut can be considered as a suitable protective strategy for improvement of diabetes side effect in testis and can have a beneficial effect on quality and quantity of sperm in diabetic males and improve fertility in these patients.
Keywords: Coconut, Sperm Analysis, Diabetes, Male Infertility

P-14: Evolution of DAZL Expression in Azoospermia Patients (Obstructive vs. Nonobstrauctive).

Hashemi MS[1, 2], Mozdarani H[1], Nasr Esfahani MH[2], Ghaedi K[2, 3]

1. Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Cellular Biotechnology, Cell Sciences Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Background: A number of patients with azoospermia/severe oligozoospermia suffers from mutations and micro deletions of somatic chromosomes as well as Y chromosome respectively. These micro deletions are frequently observed in DAZ genes family which comprises of three genes, termed as: DAZ gene localized on the Y chromosome, DAZ-like (DAZL) gene on the chromosome 3, and BOULE gene on chromosome 2. DAZ family encodes potential RNA binding proteins that are critical for germ cells development. DAZL is important for human male infertility as indicated DAZL protein transferrs from the nucleolus of spermatogonia cells into the cytoplasm during male gametogenesis. Thus the aim of present study is assessment the expression level of DAZL in testicular tissues extractions of the individuals with azoospermia in two states of obstructive and non-obstructive.
Material and Methods: To study the expression levels of DAZL, RNA quantification in testicular tissues extractions were carried out by real-time reverse transcription-polymerase chain reaction. This study included 10 men with obstructive azoospermia (OA) and 35 men with nonobstructive azoospermia (NOA) who had undergone micro- testicular sperm extraction. Nonobstructive azoospermia men were divided into three subgroups: hypospermatogeneesis (HS); maturation arrest (MA); Sertoli cell-only syndrome (SCO). The fold change expression of DAZL gene calculated by 2-ΔΔCt method. To compare the mean expression level of DAZL gene between OA and NOA men, t test has was implemeted and P values smaller than 0.05 have considered significant.
Results: Expression levels of DAZL transcript levels in testicular tissues of total nonobstructive azoospermia men was decreased compare to obstructive azoospermia. (Average mean of DAZL expression in OA= 3.2, Total NOA= 0.9, SCOS= 0.2, HS= 1.1 and MA= 1.3) but not significant $P>0.05$. Also, there was a significant difference in expression pattern of DAZl between OA and SCOS ($P<0.05$).
Conclusion: Taken together, we concluded that modulation in DAZL expression in germ cells could be involved in human spermatogenesis failure.
Keyword: Sertoli Cell-Only Syndrome, DAZL Gene, Non-Obstructive Azoospermi, Micro- TESE, Gene Expression

P-15: Environmental Exposure to Zinc, Copper, Iron, Cadmium and Lead in Testicular Tissue and Their Correlation with Serum Concentrations of Testosterone

Hassani H[1*], Rafaee A[2], Yavari M[3]

1. Department of Animal Physiology, Faculty of Agriculture and Natural Resources, Karaj Branch, Islamic Azad University, Karaj, Iran
2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Clinical Sciences, Faculty of Veterinary Sciences, University of Bu-Ali Sina, Hamedan, Iran
Email: mdfhassani@gmail.com

Background: The objective of the study was to examine the association between zinc, copper, iron, cadmium and lead concentration in Testicular Tissue and serum concentration of testosterone in ram. Chemical elements play a crucial role in male reproduction, as an unbalance in their amounts may lead to defective spermatogenesis, reduced libido, and consequently, male fertility impairment. The existing evidence for a relationship between metals exposure and hormone levels are inconsistent. Endocrine disruption leads to disorders of testicular function and thereby compromises the normal phenotypic development of male sexual characteristics, initiation and maintenance of spermatogenesis.
Materials and Methods: The blood and testes samples were obtained from 45 healthy adult rams. Testosterone concentration was measured by ELFA. The blood serum and testicular tissue samples were analyzed for the presence of metals by using an Flame Atomic Absorption Spectrometry (FAAS). Statistical analysis of results was carried out using the SAS software. The level of significance was set at $P<0.01$ and $P<0.05$.
Results: The mean concentration (standard error of the mean) of metals in testicular tissue were: zinc 212.46 ± 22.24 (μg/g), copper 16.78 ± 2.07 (μg/g), iron 150.53 ± 14.11 (μg/g), cadmium 0.15 ± 0.042 (μg/g), lead 2.64 ± 0.58 (μg/g). The mean concentration (standard error of the mean) of testosterone in blood serum was 1.63 ± 0.09 (ng/mL). The analysis showed high positive correlation between testicular lead and testosterone ($P<0.01$). Also, Significant positive correlation existed between testicular zinc and testosterone ($P<0.05$).
Conclusion: The present study showed that the zinc and lead levels in the testicular tissue of ram were significant positive correlation with testosterone. Our findings provide some evidence that low levels of metals may be associated with modest hormonal variation. Cadmium and lead have been the most studied metals in relation to altered hormone levels. The analysis confirmed that lead exposure might play a significant risk factor for fertility. Our finding of a positive association between zinc and testosterone is also consistent with previous research, as zinc is known to play a role in testicular development, sperm maturation and steroidogenesis. However, further studies are needed to better elucidate the correlation between metals and testosterone.
Keywords: Testosterone, Testicular Tissue, Heavy Metals, Ram, Chemical Elements

P-16: Cadmium and Lead in Blood Serum and Reproductive Tissues and Their Associations with Serum Luteinizing Hormone Concentration

Hassani H[1*], Rafaee A[2], Yavari M[3]

1. Department of Animal Physiology, Faculty of Agriculture and Natural Resources, Karaj Branch, Islamic Azad University, Karaj, Iran
2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Clinical Sciences, Faculty of Veterinary Sciences, University of Bu-Ali Sina, Hamedan, Iran
Email: mdfhassani@gmail.com

Background: From the publications reviewed, it appears that environmental toxicants, especially heavy metals and organic chemicals of synthetic and microbiological origins, disrupt hormone production and action in the mammalian testes. Heavy metal exposure can adversely affect male fertility and result in severe impairment of testicular functions including germ cell death and inhibition of testicular steroidogenesis. Cadmium and lead are a widespread toxic and carcinogenic metals with numerous adverse health effects in humans. In the general population, environmental exposure to cadmium occurs the consumption of contaminated food and water, and inhalation of contaminated air. Cadmium and lead have been the most studied metals in relation to altered hormone levels. This study evaluates whether lead (Pb) and cadmium (Cd) at environmental concentration are associated with serum luteinizing hormone concentration.
Materials and Methods: Forty five testes and blood samples were collected from rams and used in the study. Metals concentration in the blood serum, epididymal tissue and testicular tissue were determined by using an Flame Atomic Absorption Spectrometry (FAAS). Concentration of LH was measured by ELISA. Statistical analysis of results was carried out using the SAS software. The level of significance was set at $P<0.01$ and $P<0.05$.
Results: The mean concentrations (standard error of the mean) of metals in blood serum, epididymal tissue and testicular tissue were: lead 2.73 ± 0.06 (µg/ml), 2.42 ± 0.56 (µg/g) and 2.64 ± 0.58 (µg/g), respectively, cadmium 0.037 ± 0.15 (µg/ml), 0.03 ± 0.01 (µg/g) and 0.15 ± 0.042 (µg/g), respectively. The mean concentration (standard error of the mean) of LH in blood serum was 58.86 ± 8.09 (ng/L). There was no correlation between the metals and serum luteinizing hormone.
Conclusion: Many authors have reported heavy metal-associated detrimental effects on semen quality and fertility rates either by a direct impact on the testicular function or mediated via hormonal imbalances or toxicant-induced oxidative stress. Endocrine disruption leads to disorders of testicular function and thereby compromises the normal phenotypic development of male sexual characteristics, initiation and maintenance of spermatogenesis. We observed no significant associations between lead and cadmium and serum luteinizing hormone concentration. Several studies have investigated the relationship between exposure to heavy metals and hormone levels among occupationally exposed men. Our findings highlight the need for additional research on hormonal function in the presence of metals.
Keywords: Cadmium, Lead, Luteinizing Hormone, Reproductive Tissue, Testis

P-17: Production of Monoclonal Antibody Against Germ Cells Antigens and Localization in Adult Testis Tissue of Male Balb/c Mouse

Hemati A[1*], Modaresi MH[2], Azarnia M[1]

1. Department of Biology, Faculty of Science, Kharazmi University, Tehran, Iran
2. Department of Genetic, Faculty of Medicine, Tehran University, Tehran, Iran
Email: atefeh.hemati@gmail.com

Background: Testis is characterized for the males which is responsible for spermatogenesis and it has an special and completely unique genic expression system. Mammal s spermatogenesis is a complicated process which consists from a collection of events such as mitosis (spermatogonia), miosis(spermatocyte) and development of spermatid to spermatozoa. At first in 1969, the physiologic barrier subject was investigated in male generation system. In fact, blood testis barrier(BTB) acts as a physical barrier which prevents from arrival of immunoglobolines and leucocytes to tubules of testis. If these were destroyed, an antibody would produce. So, in recent years researches have increase on sperms as an antibody. The production of these anti sperm antibodies can cause destroying of antigen molecules and infertility. Monoclonal antibodies are produced by special cells and via a technique as "hybridoma technology". To produce hybrids between Myeloma cells and antibody producing cells, cellular fusion method is used.
Materials and Methods: In this project after immunization of mice with antigen injection, the lysis testis tissue in PBS buffer, allow to produce antibody in animals bodies after several weeks. With testing the amount of the titter of antibody by ELISA method, if the amount of antibody is sufficient, the steps of hybridoma technique would be implemented carefully. After producing the monoclonal antibody against mouse testis, characterization and analyzing of exact position of it carried out in mature mice by immunocytochemistry and immunohistochemistry.
Results: The results showed that antibody s titter in hybridoma samples from combination of Myeloma cells and B-cells with lysis testis tissue has increased in comparison with control group. Also after carrying out the immunohistochemistry technique with flouroscent antibody with contains FITC in testis tissue, the tissue sections that coloured with monoclonal antibody sample showed expression in comparison with control group in primary and secondary spermatocyte cells and spermatogonia. Furthermore there was significant increase of expression in testis tissue.
Conclusion: All findings revealed that monoclonal antibody are produced against of many proteins in testis tissue.
Keywords: Monoclonal Antibody, Germ Cells, Mice

P-18: Cerium Oxide (CeO2) Nanoparticles Affect on Spermatogenesis in Mice

Hosseinalipour E[1*], Karimipour M[2], Ahmadi A[3], Zirak Javanmard M[2]

1. Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran
2. Department of Anatomical Sciences, Faculty of Medical Sciences, Urmia University of Medical Sciences, Urmia, Iran
3. Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: elnazhosseinalipour@yahoo.com

Background: Cerium dioxide nanoparticles (CeO2NPs) are widely used as diesel additive or as promising therapy in cancerology; due to their catalytic and oxidative properties, scarce data are available on their toxicity on reproductive system. The

aim of the present study was to demonstrate the effect of Ceo2 NP on mice sperm.
Materials and Methods: Thirty four adult male mice were used. Experimental Groups (CiO2 1- CiO2 2- CiO2 3) received one of the following treatments daily for 35 days: 1, 50 and 100 mg/kg cerium oxide nanoparticles respectively intraperitoneal. Control group received only normal saline. Epididymal sperm parameters assessments were performed for evaluation of the cerium oxide nanoparticles effects on testis.
Results: Epididymal sperm parameters including sperm number, motility and percentage of abnormality were significantly changed in high dose cerium oxide nanoparticles treated mice
Conclusion: Cerium oxide nanoparticles act as testicular toxicant and further studies are needed to establish its mechanism of action upon spermatogenesis.
Keywords: Cerium Oxide, Nanoparticles, Spermatogenesis, Mice

P-19: Comparison of Free 8-isoprostane in Seminal Fluid of Healthy vs. Oligospermic Men before and after Treatment with Date Palm Pollen: A Clinical Trial

Izadi Raeini M[1], Fallahi S[2], Alamehzadeh Z[4], Malekzadeh K[3], Amini A[3], Amirzadeh Shams Sh[5]

1. Medical Biotechnology Research Center, Ashkezar Branch, Islamic azad university, Ashkezar, yazd, Iran
2. Fertility and Infertility Research Center, Hormozghan University of Medical Scines, Bandar Abbas, Iran
3. Department of Molecular Genetics, University of Ahvaz Shahid Chamran, Ahvaz, Iran
4. Department of Biology, Islamic Azad University, Arsanjan Branch, Arsanjan, Iran
5. Department of Microbiology of Islamic Azad University North Branch, Tehran, Iran
Email: zeinab_allamehzadeh@yahoo.com

Background: The exclusive cause of about 20% of couple infertilities is male disorders. In 20-40% of other case, male factors play a leading role. Oxidative stress is considered as a key factor involved in male infertility and severely damages the sperm. The free 8-isoprostane in seminal fluidis an index of DNA damage in semen. There has been solid evidence that date palm pollen (DPP) has an antioxidant property which helps to improve the quality of sperm.
Materials and Methods: In the present research, 40 oligospermic men were selected along with 10 healthy subjects. The former group were treated for 30 consecutive days with gelatin capsules containing DPP while the latter group received none. Semen samples were obtained from both groups once before the trial and once afterwards. The level of free 8-isoprostane in their semen was analyzed as an index of the severity of damage to DNA as well as an analysis of sperm parameters.
Results: The level of 8-isoprostane showed to be lower in the treatment group than the control. Sperm parameters showed to be improved after the treatment. However, the reduction of free 8-isoprostane level was not statistically significant ($P<0.05$).
Conclusion: The present findings confirmed oxidative stress as a key factor involved in male infertility. Moreover, it was indicated that DPP possesses a therapeutic potential and can positively influence fertility through reducing the severity of damage to DNA and improving sperm parameters. Although the reduced level of free 8-isoprostane was not statistically significant, it by no means rejects its effectiveness. Lengthening the duration of treatment can be recommended so as to obtain significant results.
Keywords: Infertility, Oligospermia, DPP, ROS, Free 8-isoprostane

P-20: The Protective Effect of Vitamin E on Sperm Parameters and Alteration of Malondialdehyde and Total Antioxidant Capacity in Mice following Treatment with Dexamethasone

Kamali Omidi R, Soleimani Mehranjani M
Department of Biology, University of Arak, Arak, Iran
Email: roya.kamali69@yahoo.com

Background: Dexamethasone as a common medicine is able to disturb male reproductive system through inducing oxidative stress. Vitamin E as a strong antioxidant is able to reduce the oxidative stress. The aim of this study was to investigate the effect of vitamin E (Vit. E) on the sperm parameters, Serum levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) in adult mice treated with dexamethasone.
Materials and Methods: In this experimental study, 24 adult male NMRI mice with an average weight 39 g. divided into four groups (six animals per each group) as follows: control, Dexamethasone (7 mg/kg), Dexamethasone + vitamin E (20 mg/kg) and vitamin E (100 mg/kg). Treatment was performed through intraperitoneally injection for 7days. At the end of treatment period, the blood serum was collected for measuring the level of MDA and total antioxidant potential using thiobarbituric acid method and ferric reducing ability of plasma (FRAP) assay respectively . Then the left caudal epididymis was cut to obtaine sperms for evaluating sperm parameters. Data were Statistical analysis using one-way ANOVA and Tukey's test, and the means difference was considered significantly different at $P<0.05$.
Results: A significant reduction of motility ($P<0.001$), count ($P<0.001$) and viability ($P<0.001$) of sperms were observed in the Dexamethasone group compared to control group. The mean level of MDA and TAC significantly increased and reduced in the Dexamethasone group compared to control group ($P<0.001$) respectively. The mentioned parameters were compensated in the Dexamethasone + vitamin E group to the control level ($P<0.05$).
Conclusion: This investigation reviled that treatment of mice with vitamin E for a period of 7 days can improve motility, count and viability of sperms through compensating alteration of Lipid peroxidation and total antioxidant capacity due to administration of Dexamethasone.
Keywords: Dexamethasone, Vitamin E, Malondialdehyde, Total Antioxidant Capacity, Sperm Parameters

P-21: The Report of Successfully Pregnancy after ICSI with Combined DGC/Zeta Sperm Selection Procedure in Two Couples with Six and Eight Repeated Fail IVF/ICSI Cycles

Karimi N[1*], Kouchesfehani H[1], Nasr Esfahani MH[1], Shahverdi AH[2], Chobineh H[2], Esmaeili V[3]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Reproduction and Development Department, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran

4. Infertility Center of The Iranian Red Crescent (Rouyesh), Tehran, Iran
Email: kariminazanin62@gmail.com

Background: This is the first report implementing combined density gradient centrifugation/Zeta (DGC/Zeta) sperm selection procedure in the Infertility center of the Iranian Red Crescent (Rouyesh) for two couples with six and eight previous intra cytoplasmic sperm injection/ *in vitro* fertilization (ICSI/IVF) failure cycles.
Materials and Methods: Semen analysis was carried out according to World Health Organization criteria. Morphology, Protamine deficiency and DNA fragmentation were assessed by papanicolaou chromomycin A3 (CMA3) and TUNEL assay staining, respectively. Patient was counseled regarding DGC/ Zeta sperm preparation procedure.
Results: For two patients, 10 oocytes were injected with combined DGC/Zeta sperm preparation which resulted in 90% fertilization rate and six embryos with good quality. Two embryos were transferred on day three. For two Patients, Singleton pregnancy two healthy girl and boy baby delivered with cesarean section.
Conclusion: Result of this case report may open the horizon for further evaluation Zeta of sperm selection procedure for couples with repeated ICSI/IVF failure.
Keywords: Intracytoplasmic Sperm Injections, Density Gradient Centrifugation, Fertilization, Pregnancy

P-22: Protective Effect of Mesenchymal Stem Cells and Crocin following Torsion-Detorsion on Testis of Mice

Khaneshi F

Department of Basic sciences, Veterinary Medicine, Urmia, Iran
Email: f.khaneshi@yahoo.com

Background: Cell therapy is a promising treatment method now a day. The purpose of this study was the evaluation of protective effect of mesenchymal stem cells (MSCs) and Crocin (Cr), following torsion-detorsion injuries in testis tissue.
Materials and Methods: In this study 40 male mice were randomly divided into 4 groups; Mice in all groups were undergoing testicular torsion-detorsion (T-D). The first group (T-D) received pbs, second group (T-DM) was injected MSCs(5×10^5 in 10µL) in to rete-testis. Third group (T-DCr) received Cr 200mg/kg (IP), The fourth group(T-DMCr) was injected MSCs and Cr. After 35 days testicular tissue was sampled, and paraffin sections were prepared and staind with HandE method. Histomorphomerical study was performed on spermatogenesis and spermiogenesis parameters in seminiferous tubules. Data analized by SPSS software and ANOVA statistic method and Tukey post hoc test. Significant differences were described as $P<0.05$.
Results: The mean number of Primary spermatocytes, spermatogonia and Spermatide in all groups were significantly increased in comparison to (T-D) group ($P<0.05$). Also result showed that the aforementioned parameters in T-DCr and T-DM groups were significantly lower in comparison T-DMCr. Torsion-Detosion caused a significantant increase in interstitial space in T-D group in comparison with other groups ($P<0.05$).
Conclusion: Co-administrating of MSCs and Cr ameliorated the stress oxidative induced by T-D, better than the administrating of them separatelly ($P<0.05$).
Keywords: Mesenchymal Cell, Crocin, Testis, Torsion

P-23: Effect of Alpha Lipoic Acid (ALA) Antioxidant on Human Sperm Motility and Viability after Incubation in 37°C and 3h

Khayamabed R[1*], Taherian S[1], Tavalaee M[1], Nasr Esfahani MH[1, 2]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Background: Alpha-lipoic Acid (ALA) is known to be a natural antioxidant and is soluble in water and fat. Several studies have shown that the process of washing sperm due to removal of seminal plasma, reduces the antioxidant defense capacity and causes oxidative stress by increased levels of oxygen free radicals and decreased sperm motility and viability. Therefore, the aim of the present study was to evaluate the effect of ALA on the motility and viability of human spermatozoa during 3h at 37°C.
Materials and Methods: In this study, 34 normozoospermic semen samples referring to Isfahan Fertility and Infertility Center were collected. Each semen sample was divided two portion, one portion was washed with sperm washing media and considered as "control" group and other portion was processed with density gradient centrifugation (DGC) procedure. Processed sperm with DGC was divided two portion; 1) sperm sample was incubated with alpha-lipoic acid antioxidant for 3 hours at 37 ° C and 2) sperm sample was not incubated with alpha-lipoic acid antioxidant for 3 hours at 37° C. Then, sperm motility and viability were assessed by CASA (Computer-assisted sperm analysis) system and Eosin-Necrosin staining on each portion, respectively.
Results: Our results showed that percentage of sperm motility in the treatment group (58.39 ± 1.59) had a significant increase compared to the control group (44.95 ± 1.73; $P=0.045$). Also, percentage of sperm viability was significantly higher in treatment group (66.54 ± 2.84) than the control group (50.26 ± 3.35; $P=0.004$).
Conclusion: The result of the current study clearly shows that adding alpha-lipoic acid antioxidant to processed sperm can lead to maintain some sperm parameters such as motility, and viability during time.
Keywords: Alpha-Lipoic Acid, Sperm Motility, Sperm Viability, DGC

P-24: Screening and Detecting Chlamydia Trachomatis Infection in Semen Samples of Infertile Men Referring to Royan Institute

Khoshakhlagh A[1*], Salman Yazdi R[1, 2], Navab-Akbar FT[3], Ghaheri A[4], Sadeghinia SH[5], Dadkhah F[2]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Microbiology, Islamic Azad University, Naein Branch, Naein, Iran
3. Department of Microbiology and Virology, Isfahan University of Medical Science, Isfahan, Iran
4. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Biomolecular and Biomedical Sciences, School of Health Life

and Science, Giasgow Caledonian University, Glasgow, United Kingdom
Email: amin.khoshakhlagh60@gmail.com

Background: Chlamydia trachomatis (CT) with adverse ramifications on sperm quality parameters can often lead to infertility in men. The main purpose of the present research was to determine the screening and detecting CT infection in semen samples of infertile men referring to Royan Institute.
Materials and Methods: In this cross-sectional study, 465 patients referring to the clinical laboratory of Royan Institute were randomly chosen for primary screening and detection of the presence of CT among whom 93 samples were normozoospermia (Asymptomatic) and other 372 had abnormal parameters (Symptomatic) in semen analysis. ELISA test was conducted as the screening test to trace the presence of anti-CT IgA in the patients' seminal plasma. 62 samples (32 symptomatic and 30 asymptomatic) with higher results in ELISA were selected as the case group and 34 asymptomatic samples with negative results were randomly selected as the control group for confirmatory test. The sperm DNA was extracted to confirm the presence of CT. PCR assay was recruited to confirm the serological results by using species-specific primers for amplification of CT genome. Data statistical analysis was made by using SPSS 16.
Results: 62 out of 465 samples had Optical Density (OD)>0.200 in ELISA screening test selected as the case groups for the molecular assay. 34 asymptomatic samples with OD<0.200 in ELISA test were selected as the control group, as well. In the case groups, 4 out of 32 symptomatic samples (12.5%), and 1 out of 30 asymptomatic samples (3.3%) indicated positive results in the PCR. No PCR positive sample was observed in the control group. Furthermore, the comparison of two symptomatic and asymptomatic groups showed that there was no significant difference between the age (P=0.253) and the patients' semen volume (P=0.447). The ultimate results revealed that considering OD>0.400 as the ELISA Positive, the prevalence of CT-ELISA Positive in symptomatic and asymptomatic infertile patients with Iranian nationality were 0.019 (7 of 372) and 0.021 (2 of 93), respectively.
Conclusion: CT will lead to pelvic inflammatory disease (PID) and infertility if not diagnosed and treated. Screening of infertile men who do not show any clinical symptoms look inevitable and can be considered as a part of the program of sexually transmitted disease (STD) control. In conclusion, the anti-CT IgA ELISA test could be introduced as a suitable tool for screening purpose in the seminal plasma of infertile men.
Keywords: Chlamydia Trachomatis, Infertile Men, ELISA, PCR, Screening

P-25: Study of Diazinon Poison Level in Semen Liquid and Its Effect on The Sperm Parameters in Infertile Men in Babol City

Kiani S[1], Shariatzadeh SM[1], Jorsaraei SGh[2], Parsian H[3], Tabari M[3]

1. Department of Anatomy, Babol University of Medical Sciences, Babol university, Babol, Iran
2. Department of Biology, Arak University of Science, Arak University, Arak, Iran
3. Department of Clinical Biochemistry, Babol University of Medical Sciences, Babol university, Babol, Iran
Email: samanekiani@yahoo.com

Background: Diazinon (DZN) is an organophosphorus insecticide with a prominent toxicity on many body organs.The aim of this study was to investigate the Diazinon poison level in semen liquid and its effect on the sperm parameters.
Materials and Methods: 90 Adult males (18-52 years old) were divide into three groups (n=30). Group 1 (including Infertile men who exposed to Diazinon), group 2 (including Infertile men who didn't expose to Diazinon) and group 3 as the control (including Fertile men who didn't expose to Diazinon). semen insecticide level was analyzed using high-performance liquid chromatography (HPLC) method. Some parameters such as seminal volume ،sperm concentration ،total sperm number، sperm morphology، sperm motility، sperm viability, plasma levels of malondialdehyde (MDA) and testosterone levels were analyzed. Data were analyzed using one-way ANOVA and means were considered signification different at P<0.05.
Results: Significantly decreased the volume of the semen، sperm concentration، total sperm number، sperm morphology، sperm motility، sperm viability and plasma testosterone level, and also significantly increased in the MDA level was seen in the groups 1 and 2 in compared with the control group (P<0.05). Also, decreased the volume of the semen، sperm concentration، total sperm number، sperm morphology، sperm motility، sperm viability and plasma testosterone level, and increased in MDA level was observed in the group of the infertile men who exposed to diazinon in compared with the group of the infertile men who didn't expose to Diazinon.
Conclusion: This study showed that Diazinon could effect on semen quality and male fertility in humans. Therefore, application of DZN should be limited to a designed Program.
Keywords: Diazinon, Organophosphorus Insecticide, Human, Semen Liquid, Sperm Parameterse

P-26: Protective Effects of Royal Jelly Against Heat Stress Evoked Epididymal Sperm Impairments in Rats

Mahdivand N[1*], Nejati V[1], Najafi Gh[2], Shalizar Jalali A[2], Rahmani F[1]

1. Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: n_mehdivand@yahoo.com

Background: Higher temperatures can lead to increased testicular metabolism resulting in spermatogenesis disruption. The aim of this study was to evaluate the protective effects of royal jelly (RJ) against epididymal sperm impairments following heat stress induction (37, 39 and 43 °C for 20 min) in male rats.
Materials and Methods: 40 adult male rats were randomly categorized in to the 8 groups (n=5) including control, RJ, 43°C, 39°C, 37°C, 43°C+RJ, 39°C+RJ and 37°C+RJ. Royal jelly was administered at a dose of 100 mg/kg by oral gavages. After 48 days, epididymal sperm characteristics were analyzed in all experimental groups.
Results: The data revealed that epididymal sperm motility and viability decreased significantly (P<0.05) in heat stress group compared to control and RJ groups. Interestingly, RJ co-administration significantly ameliorated above-noted parameters (P<0.05). There was no significant (P<0.05) difference between RJ and control group.
Conclusion: The present results demonstrate that RJ can de-

crease heat stress induced epididymal sperm disorders in rats.
Keywords: Heat Stress , Royal Jelly, Sperm, Rat

P-27: Hormone Profiling as A Biomarker Predictive in Non-Obstructive Azoospermia Men

Mardi Mamaghani A[1*], Hosseini J[2], Moslemi E[1]

1. Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: jhosseinee@gmail.com

Background: Infertility is clinically defined as failure of a couple to conceive after one year of regular sexual intercourse and occurs in both males and females for various reasons. About half of the infertility causes is due to male factors which up to 10% are due to azoospermia, or lack of sperm in the ejaculate. Azoosperima is divided into two types: non-obstructive azoospermia (NOA) and OA (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 to allow fertility. As TESE is an invasive diagnostic technique for predicting the presence or absence of spermatozoa in the testis a non-invasive biomarker seems critical. In addition, 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. Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and Testosterone are three main hormones evaluated during infertility treatments. FSH and LH secreteed from the gonadotropes are controlled by the hypothalamic decapeptide and testosterone is secreted from Leydig cells. So finding a non-invasive and hormonal biomarker is essential
Materials and Methods: The study population included 42 infertile non-obstractive azoospermia men referred to Royan institute. Based on the results of their biopsy, patients were categorized into two groups: TESE+ and TESE-. Blood samples were collected from patients before TESE surgery and separation of serum and measurement of the testosterone, LH and FSH hormones were performed by enzyme-linked immunosorbent assay (ELISA) technique. SPSS software version 18 and Chi- square test, Independent t test and Pearson correlation coefficient statistical tests were used for data analysis. The P value ≤ 0.05 was considered significant.
Results: The present study showed that FSH and LH hormone levels were relatively significant between TESE+ and TESE- patients (P=0.07 and P=0.08 respectively) and their hormonal level were higher in TESE- compared to TESE+ in the blood samples. There was no significant difference in the testosterone mean between the two groups mentioned (P=0.66).
Conclusion: In this limited observation, no significant differences were seen for the levels of FSH and LH hormones between TESE+ and TESE- group patients. Therefore, if absolute significant results achieves by evaluating a larger population of non-obstractive azoospermia patients these hormones can be used as predictive biomarkers instead of the invasive diagnostic technique routinely used.

Keywords: LH, FSH, Non-Obstructive Azoospermia, Testicular Sperm Extraction, ELISA

P-28: The Protective Effect of Quercetin on Sperm Parameters and Serum Levels of Malondialdehyde and Total Antioxidant Capacity in Mice Treated with Dexamethasone

Mohammadi M
Department of Biology, University of Arak, Arak, Iran
Email: mohammadi.maryam23@yahoo.com

Background: Dexamethasone is a synthetic glucocorticoid which has a negative impact on male fertility .Quercetin is a powerful antioxidant with a compound flavonoid. The purpose of this investigation was to evaluate the protective effect of Quercetin on the adverse effect of dexamethasone on sperm parameters and Serum levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) in adult mice following treatment with dexamethasone.
Materials and Methods: Adult male NMRI mice (39 g) were divided randomly into 4 groups (n=6), control, dexamethasone (7 mg/kg/day), dexamethasone + Quercetin (50 mg/kg/day) and Quercetin. After7 days of intra peritoneal injection treatment, blood samples were taken for measuring total antioxidant capacity (by Frap test) as well as levels of malondialdehyde (MDA) using thiobarbituric acid method. Then the left caudal epididymis was cut in the Hams F10. Released spermatozoa were used to analyze sperm parameters. Data were analyzed using one way ANOVA and means were considered significantly different at $P<0.05$.
Results: A significant decrease in the mean motility ($P<0.001$), count ($P<0.001$) and viability ($P<0.001$) of sperms was found in the dexamethasone group compared to the control ones .Mean motility and count of sperms increased in the dexamethasone + Quercetin group to the control level ($P<0.05$), while sperm viability ($P<0.001$) was increased in this group in comparing with dexamethasone group.A significant increase of MDA and decrease of TAC was observed in the dexamethasone group compared to the control group ($P<0.001$). A significant decrease of MDA and increase of TAC was seen in the dexamethasone + Quercetin group compared to the dexamethasone group ($P<0.001$).
Conclusion: Our results indicated that Quercetin may be useful in reducing dexamethasone induced adverse effects on sperm parameters and malondialdehyde and total antioxidant capacity through inducing oxidative stress.
Keywords: Quercetin, Dexamethasone, Mice, Sperm Parameters

P-29: Beneficial Effects of N- Acetylcysteine on Mancozeb-Induced Reproductive Toxicity in Male Mice

Mohammadisardoo M[1*], Mandegary A[2], Nabiuni M[1], Nematollahi Mahani N[3], Amirheidari B[4]

1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
2. Department of Pharmacology and Toxicology, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
3. Department of Biotechnology, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran
4. Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
Email: alimandegary@yahoo.com

Background: In recent years, reproductive toxicity has been a topic of increasing interest and concern. Environmental exposure to potential toxicants may cause serious health risks including fertility and reproductive function. Mancozeb (MZB), a Mn/Zn-containing ethylene bisdithiocarbamate (EBDC), has demonstrated nearly seventy years of fungicidal efficacy in a wide range of agricultural and industrial applications. It is reported that MZB can affect the male reproductive system by enhancing oxidative stress. N-acetylcysteine (NAC), a potent antioxidant and US-FDA approved drug could be involved in scavenger of free radicals. In the present study we evaluated NAC protective effect against MZB-induced oxidative stress.
Materials and Methods: To follow- up current study, mature male mice treated with vehicle, MZB alone (500mg/kg, orally. for 40 consecutive days), NAC (200 mg/kg, i.p.), NAC plus MZB (MZB given 1 hour post NAC). Assessment of testicular toxicity was done by studying changes in histology parameters.
Results: Observations demonstrated that seminiferous tubules are normal in the control and NAC group. Histopathological evaluations clearly indicated severe degeneration, decreased number of germ cells in MZB-exposed testes. Interestingly, in NAC plus MZB group, NAC alleviated MZB-induced histopathological damage in the testes.
Conclusion: In summary, result of the current study suggests that NAC supplementation can be useful in testis oxidative injury caused by the mancozeb.
Keywords: Mancozeb, N-Acetylcysteine, Oxidative Stress, Testis Tissue

P-30: Effect of Aerobic Exercise on Sperm Parameters in Male Mice C57BL/6

Nematollahi A[1*], Tavalaee M[1] Nasr Esfahani MH[1, 2]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Background: Sperm production and quality can be affected by aspects of lifestyle such as exercise. Effects of exercise on male reproductive function are related to the severity and period of physical activity and individual's fitness. It was demonstrated that the number of hours per week that men doing physical activity was directly linked with sperm concentration and sperm count; While inactivity had a negative correlation with these results. The aim of this study was to determine the effect of aerobic exercise on sperm parameters.
Materials and Methods: 45 male mice C57BL/6 were used for this study and divided into two groups. Study group contained 23 mice which underwent specific exercise protocol and they were compared with a control group which did not any extra activity (N=22). Animals were sacrificed by using a standard protocol and, epididymis were isolated. Then, sperm concentration and motility were assessed by light microscopy and, sperm morphology were stained by eosin-nigrosin and evaluated.
Results: Our results showed that the sperm concentration in the exercise group (38.07 ± 1.89) had a significant increase compared to the sedentary group (33.14 ± 1.46) (P=0.013). Also, percentage of sperm motility was significantly higher in study group (61.32 ± 3.83) than the sedentary group (49.25 ± 2.33) (P=0.00). Percentage of abnormal sperm morphology, abnormal head, neck and tail were not significant between two group, except percentage of abnormal head morphology (P=0.011).
Conclusion: The result of the current study clearly shows that moderate aerobic exercise can lead to improvement of some sperm parameters such as concentration, and motility.
Keywords: Exercise, Sperm Motility, Sperm Concentration, Sperm Morphology

P-31: Ceftriaxone-induced Toxicity in Testis of Mice: Histological Evidences

Pirdehghan HR[1], Bonyadi F[2*], Hasanzadeh Sh[2], Najafi Gh[2]

1. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, Urmia, Iran
2. Department of Basic science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: farzane.bonyadi@yahoo.com

Background: Ceftriaxone is a third-generation of cephalosporin antibiotic that has broad spectrum activity against bacteria. Recently the adverse effects of this drug have been reported on the reproductive system. The aim of this study was to evaluate adverse effects of ceftriaxone on testicular tissue of adult male mice.
Materials and Methods: 40 adult male mice were randomly divided into 5 groups, including a control received normal saline. The 1 and 2 experimental groups were received ceftriaxone at 20 mg/kg and 50 mg/kg for 7 days respectively. The 3 and 4 groups were received 20 mg/kg and 50 mg/kg for 45 days respectively. Following tissue preparation processes and general staining, the histological and histomorphometric studies were performed. The data were analyzed by one way ANOVA and Tukey, s test. Significant level was considered $P<0.05$.
Results: The histological assessment of experimental groups represents the degeneration of some seminiferous tubules, reduction of spermatogenesis cell series and supporting cell populations and incoherence of them. In the interstitial tissue were seen changes such as edema, cluttering of lymphoid aggregates and scattered groups of leydig cells. Significant reduction ($P<0.05$) were seen in mean diameter of the seminiferous tubules, testicular capsule, thickness of epithelium, distribution of leydig cells compared to the control group. Distribution of lymphocytes showed a significant decrease in some groups.
Conclusion: Ceftriaxone impaired testicular tissue structure, including impaired structure of seminiferous tubules and interstitial tissue, also loss of consistency in the population of spermatogenesis cell series.
Keywords: Ceftriaxone, Testis, Histology, Histomorphometry

P-32: Association of Hyaluronic Acid Binding Assay with Sperm Parameters in Teratoasthenozoospermic Patients and Fertile Donors

Rahimizadeh P[1*], Rezaei Tobraggaleh T[1], Rashki Ghaleno L[1], Esmaeili V[1], Sharbatoghli M[1], Maroufizadeh S[2], Eftekhari-Yazdi P[1], Mousavi M[1], Shahverdi AH[2]

1. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: Selection of high quality spermatozoa in assisted reproductive techniques(ART) is very important to improve clinical outcomes. Currently, the different advanced techniques such as hyaluronic acid binding assay (HBA) use for sperm selection. Even though huge studies supported the ability of HBA for prediction of some sperm characteristics, its potency to predict classical and intracellular sperm parameters in teratoasthenozoospermic men still remains unknown. Thus this study was performed to determine the correlation of HBA with conventional sperm parameters and DNA fragmentation in teratoasthenozoospermic males and fertile donors.
Materials and Methods: The semen samples were obtained from 80 males (40 fertile donors and 40 teratoasthenozoospermic infertile patients). Following liquefaction, classical sperm parameters such as concentration, motility and morphology were assessed according to the latest WHO guidelines by computer assisted sperm analyzer (CASA) and Papanicolaou staining. Then the HA-binding assay and DNA fragmentation were carried out by HBA slides and sperm chromatin structure assay (SCSA) respectively. The correlation were analyzed by the Pearson's coefficient test and the statistical SPSS software version 22.0 was used.
Results: None of features evaluated, either in fertile controls or infertile patients, was significantly correlated with HBA except normal morphology in patients group (r= 0.315, P value 0<0.05). However, when the samples from fertile controls and infertile patients were analyzed collectively as one common group, the HBA showed a significant positive relationship with concentration and normal morphology (r= 0.196, P value 0<0.05) whereas it had only a significant negative correlation with DNA fragmentation (r=-0.220, P value 0<0.05). HBA also did not indicate any association with motion characteristics.
Conclusion: The present study reports that the HBA is a reliable test for prediction of sperm morphology and DNA integrity. Therefore, using HBA could increase the chances of choosing the sperm with good morphology and nuclear integrity in order to improve ART outcomes particularly in teratoasthenozoospermic patients.
Keywords: Hyaluronic Acid Binding Assay, Conventional Sperm Parameters, DNA Fragmentation, Teratoasthenozoospermic Patient

P-33: Assessment of DNA Fragmentation in Normozoospermic Men with Unexplained Infertility

Rahimizadeh P[1*], Rezaei Tobraggaleh T[1], Esmaeili V[1], Sharbatoghli M[1], Maroufizadeh S[2], Eftekhari Yazdi P[1], Shahverdi A[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: The evaluation of spermatozoa DNA fragmentation has emerged as an important biomarker for male factor infertility. According to the considerable amounts of data, sperm chromatin integrity has been related to different types of infertility, including unexplained infertility. Nevertheless, it is still unclear whether the level of DNA damage in unexplained infertile males is high or not. Therefore, this study carried out to assess sperm DNA integrity in unexplained infertile patients.
Materials and Methods: The semen samples were collected from 40 fertile controls and 40 unexplained infertile normozoospermic men. Subsequently, liquefied semen specimens were assessed for concentration, motility and morphology according to the latest WHO manual. After initial evaluation, sperm DNA fragmentation was conducted by sperm chromatin structure assay (SCSA). All values were expressed as mean ± SD. Comparison of means was performed by one-way ANOVA, followed by the Duncan post-hoc test. Statistical analysis was carried out using SPSS software, Version 22.0.
Results: Among all the parameters evaluated, total and progressive motility in unexplained infertile patients were significantly higher than fertile group (total 73.64 ± 1.91 vs. 65.89 ± 1.84 and progressive 67.30 ± 2.35 vs. 58.38 ± 2.13, P value 0<0.05). However, the total sperm count of fertile donors was significantly higher compared to the patient's group (96.75 ± 14.89 vs. 69.76 ± 3.97, P value 0<0.05). In addition, no significant differences were observed in normal morphology between the fertile and infertile men (P value 0>0.05). Finally, the spermatozoa from the unexplained infertile males showed the significantly increased DNA fragmentation in contrast to the other group (8.39 ± 0.89 vs. 5.13 ± 0.60, P value 0<0.05).
Conclusion: This study indicates that DNA fragmentation seems to play a role in unexplained male infertility. Furthermore, using DNA fragmentation level for distinguishing unexplained male infertility is more accurate than the conventional parameters. Thus DNA integrity has clinical importance as a possible diagnostic and prognostic tool in the evaluation of male unexplained infertility.
Keywords: Unexplained Male Infertility, DNA Fragmentation, Sperm Chromatin Structure Assay

P-34: Mouse Maternal Omega-3 Dietary Fatty Acid with or without Vitamin E Effects on The Offspring's Sperm Kinematic Characteristics

Ghasemi ZS[1*], Ramezani M[1], Alizadeh A[2]

1. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: sadatghasemi@yahoo.com

Background: Vitamin E protects PUFA (polyunsaturated fatty acid) in phospholipids of membrane against peroxidation. We investigated the influence of maternal dietary fish oil (omega-3 source) with and without vitamin E on offspring' sperm parameters.
Materials and Methods: Thirty-six female mice in 6 groups fed different diets adlibitum during one week before mating to weaning day. The dietary groups were standard diet (control;C, 50 IU vit.E/kg diet), CLF and CHF groups which consumed 15 and 30 mg fish oil/100g of C diet, respectively. Moreover, a group received vitamin E supplemented diet (E, 125IU vit.E/kg diet), ELF and EHF groups received 15 and 30 mg fish oil/100 g E diet, respectively. All maternal diets contain 3 percent oils which sunflower oil replaced with fish oil. The epididymal spermatozoa were retrieved and sperm parameters were evaluated by CASA (computer- assisted sperm analysis). All data was analyzed by SAS.
Results: Concentration of sperm increased (P<0.05) in vita-

min E group. Total motility was higher (P<0.05) in CHF (47 ± 4.22%) and EHF (63 ± 3.56%) than C (41 ± 5.07 %). Progressive motility of sperm affected (P<0.05) by high fish oil added to vitamin E diet (22 ± 3.81% vs. 42 ± 4.69% for C and EHF, respectively). Non-progressive sperms did not differ. The proportion of immotile sperms decreased (P<0.05) when fish oil inclusion in vitamin E diets. Average values of sperm speed; VCL (51 ± 6.91vs. 96 ± 7.25µm/s), VSL (12 ± 2.47vs. 39 ± 4.81µm/s) and VAP (21 ± 3.42 vs. 53 ± 4.45µm/s) (C vs. EHF) were higher when mothers fed high fish oil and vitamin E diet (P<0.05).

Conclusion: Maternal dietary fish oil and vitamin E increased sperm kinematic parameters and they were approximately 2.5 folds greater in combination group (EHF) than others. Therefore, it is necessary to be considered these effects on male offsprings' reproductive when some nutrients recommended during pregnancy and breastfeeding regarding other objectives such as mental and visual function.

Keywords: Maternal Nutrition, Fish Oil, Vitamin E, Sperm

P-35: A Molecular Study of Testis after Experimentally Induced Varicocele in Rat

Sadeghi N[1*], Tavalaee M[1], Nasr Esfahani MH[1, 2]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Background: Varicocele is an abnormal enlargement of the pampiniform plexus in the spermatic cord. Adverse effects of varicocele on spermatogenesis can cause alterations in sperm concentration, motility, and morphology. Although the varicocele has been studied for many years, the pathophysiology of a varicocele still remains obscure. Autophagy is an evolutionarily conserved self-degradation process which provides a membrane dependent mechanism for the sequestration, transport, and lysosomal turnover of subcellular components, including proteins and organelles. In this study, we aimed to demonstrate the relationship between varicocele and a type of cell death called Autophagy.

Materials and Methods: A total of 10 Wistar rats were randomly divided into two groups: surgically induced left varicocele and untreated controls. Two months after surgery, animals were euthanized with ether. Then left testes were collected, processed, and stained with Immunohistochemical procedure.

Results: The results showed Atg7 protein was expressed in all types of spermatogenic cells of seminiferous tubules in varicocele group compared with the control group.

Conclusion: Our results demonstrate that following varicocele induction, autophagy increases in cells of seminiferous tubules especially in germ cells. This imply that autophagy might be another potential mechanism participating in germ cell death after heat stress in varicocele condition.

Keywords: Varicocele, Autophagy, Rat, Immunohistochemical

P-36: Effect of Ghrelin on Total Antioxidant Capacity, Lipid Peroxidation, Sperm Parameters and Fertility in Mice Against Oxidative Damage Caused by Cyclophosphamide

Salimnejad R*, Soleimani Rad J, Mohammad Nejad D, Roshangar L

Department of Anatomical Science, Faculty of Medicine, Tabriz University of Medical Sciences, Tazriz, Iran
Email: Soleimanirj@yahoo.com

Background: Cyclophosphamide (CP) is a drug used for chemotherapy and immune-suppressive in organs transplantation. Despite many clinical implications in the treatment of cancer, this drug has toxic effects on body organs, especially reproductive system. Functional change in the male reproductive system is one of the most important side effects that may lead to infertility. This study aims to evaluate the antioxidant effect of ghrelin against damages caused by oxidative stress induced by CP.

Materials and Methods: In this experimental study 40 mice were randomly divided into 4 groups: 1) control, 2), cyclophosphamide, 3) cyclophosphamide + ghrelin and 4) ghrelin. Cyclophosphamide (100 mg/kg body weight), once a week, and ghrelin (80 µg/kg body weight), daily, were administrated intraperitoneally for 5 weeks. After 5 weeks, the epididymides were removed and lipid peroxidation, total antioxidant capacity, sperm parameters and the percentage of sperm fertility were examined. *In vitro* fertilization (IVF)technique was utilized to study the sperm's fertilization potency in the studied groups.

Results: In mice exposed to CP, the number and viability of sperms, as well as total antioxidant capacity decreased significantly (P<0.05) and the number of abnormal sperms and MDA levels indicated a significantly increase (P<0.05). In addition, the fertility rate decreased in this group, while the use of ghrelin significantly improved the above disorders in the treatment group (P<0.05).

Conclusion: The findings of this study showed that ghrelin reduces negative effects caused by CP in sperm parameters and increases the fertility.

Keywords: Cyclophosphamide, Oxidative Stress, Ghrelin, Sperm Parameters, Fertility

P-37: Evaluation of DNA Fragmentation in Alpha Lipoeic Acid-Treated Male Rats with Experimental Varicocele Induction

Shaygannia E[1*], Akhavanfarid GR[1], Nasr Esfahani MR[2, 3], Tavalaee M[2]

1. Research center of Raha Pharmaceutical Company, Royan Institute for Biotechnology, Isfahan, Iran
2. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Isfahan Fertility and Infertility Centre, Isfahan, Iran
Email: mh.nasr-esfahani@rooyan institute.org

Background: Varicocele is known as the main cause of male infertility. A close linkage have been assessed between its pathogenesis and oxidative stress. Nowadays researchers are investigating on the relation between male infertility and oral intake of some antioxidants such as vitamin E and vitamin C. Alpha lipoeic acid (ALA) is one of the new antioxidant nutrition which recently noticed that is highly used in diabete, multiple scleorosis, alzheimer and several other diseases . Surprisingly; it can be solved in both hydroid and lipoid environments despite of other kind of vitamins. Accordingly, in the present study we tried to investigate effects of ALA on sperm DNA fragmentation in rats with experimentally induced varicocele.

Materials and Methods: In the present study, 40 adult male

Wistar rats were divided into 4 groups of 10 animals each. Control and varicocele-induced groups were considered as group I and II, respectively. Consequently, group III and IV were contained rats received and did not receive alpha lipoeic acid for 2 months after inductions of varicocele as well. Then, sperm DNA assayed by acridine orange in spermatozoa which were ubtained from epididyme of killed rats.
Results: Acridin orange positive spermatozoa were significantly lower (P.value= 0.034) in VI.ALA+ group in comparison of VI.ALA- group which indicates lower rate of DNA fragmentation in the drug-treated group.
Conclusion: For the first time, we evaluate the antioxidant effect of ALA in varicocele-induced rats. We concluded that ALA has a strong potential in neutralizing oxidant effect of ROS-induced by varicocele on sperm DNA damage in male rats.
Keywords: Varicocele, Antioxidant, ALA, DNA Fragmentation

P-38: Study of Association between UBE2B Variants with Susceptibility to Idiopathic Idiopatic Infertility in North Iranian Male Population

Siasi E[1*], Safai MS[2]

1. Department of Genetic, Center of Science, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Microbiology, Center of Science, North Tehran Branch, Islamic Azad University, Tehran, Iran
Email: emi_biotech2006@yahoo.ca

Background: Infertility is a major clinical problem that involved about 10 to 15 percent of couples worldwide. Male infertility include 40 to 50 percent of all infertility cases and 37-58% of male infertility are unknown, as idiopathic male infertility. This disease is caused by the interaction between genetic and environmental factors. UBE2B gene and its variations is one of the genetic factors in idiopathic male infertility. In this research was studied relation of T293G and A20016G polymorphism in UBE2B genes with the possibility of male infertility in Northern Iranian population.
Materials and Methods: For this study, samples from 60 fertile men and 60 infertile men were selected. Then DNA was extracted from samples. Genotype and allele frequencies of the variants were determined by PCR- RFLP.
Results: Statistical analysis in this study showed no significant association between patient and control groups for T293G (P=0.66) and A20016G (P=0.52) SNPs, in UBE2B gene.
Conclusion: The results were indicated that two SNPs was not associated with idiopathic male infertility in Northern Iranian male population.
Keywords: Idiopathic Male Infertility, Polymorphism, UBE2B gene, PCR-RFLP

P-39: Sperm PLCζ Protein and DNA Fragmentation Status in Globozoospermic Men

Tavalaee M

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: tavalaee.royan@gmail.com

Background: The sperm 'round head' defect, also known as globozoospermia, is a rare type of teratozoospermia with very low incidence (<0.1%) among infertile individuals. Failed fertilization due to failure in oocyte activation after intra-cytoplasmic sperm injection (ICSI) was reported in these individuals. a sperm-specific phospholipase C (PLCζ) as main sperm factor involved in oocyte activation is expressed in elongated spermatids and located in the post-acrosomal sheath of sperm perinuclear theca where fusion of sperm membrane with oolemma occurs. Considering previous studies showed influence of sperm DNA damage on fertilization and early embryonic development, we aimed to assess two main sperm factors including sperm DNA fragmentation and PLCζ protein in infertile men with globozoospermia.
Materials and Methods: In this study, semen samples were collected from 30 globozoospermic men and 30 fertile men. Sperm parameters (concentration, motility, morphology) were assessed according to World Health Organization (2010). Fluorescent microscope and western blot techniques were used for assessment of sperm DNA fragmentation and PLCζ protein, respectively.
Results: Quality of sperm parameters (concentration, motility and normal morphology) were significantly lower in infertile men with 100% round headed spermatozoa and mean expression of sperm PLCζ protein was also significantly lower in infertile men with globozoospermia compared to fertile individuals (P<0.05). In addition, percentage of sperm DNA fragmentation was significantly higher in in infertile men with globozoospermia compared to fertile individuals (P<0.01).
Conclusion: The result of this study show high sperm DNA fragmentation and low expression of PLCζ protein in infertile individuals with globozoospermia. Therefore, assessment of sperm DNA fragmentation and PLCζ as index for fertilization potential of a semen sample in men with globozoospermia may define individuals who are candidates for artificial oocyte activation (AOA) and may avoid failed fertilization post ICSI.
Keywords: Globozoospermia, PLCζ, DNA Fragmentation, Failed Fertilization, ICSI

P-40: Protective Effects of Hydro-Ethanolic Extract of Stachys lavandulifolia Vahl against Testicular Torsion Induced Damage

Yari S*, Karamian R, Asadbegy M, Khabazian Z

Department of Biology, Faculty of Basic Science, Bu-Ali Sina University, Hamedan, Iran
Email: yarisiamak@yahoo.com

Background: Testicular torsion is an emergency condition occurring primarily in children. Testicular torsion results in ischemic condition in testis tissue. Studies have shown that formation of free radicals during ischemic period has important role in pathological changes of tissue. Supplementation with natural anti-oxidative factors, can prevent harmful effects of ischemia induced free radicals. In this study we aimed to examine the protective effects of hydro-ethanolic extract of SL against ischemia-reperfusion (I/R) induced testicular damage.
Materials and Methods: Twenty-four male Wistar albino rats were randomly divided into four equal groups as follows: group I, control group (received only saline); group II, I/R group; group III, I/R + SL group; Group IV, SL group. The ischemia period was 2 hours. Saline and SL administered daily by gavage. At the end of experimental period, testes were removed for histological and biochemical analysis. All treatment were performed orally via gavage. Total protein level, Superoxide

dismutase (SOD) activity, Malondialdehyde (MDA) and H_2O_2 levels were evaluated for biochemical analysis. Histological examination were conducted for measuring of seminiferous tubules diameter.
Results: MDA and H_2O_2 levels in I/R group significantly were higher than control, I/R+SL and SL groups. In addition, total protein level, sperm counts, SOD activity were significantly decreased in I/R group. However, pretreatment with SL extract resulted in significant improvement in MDA, H_2O_2, total protein level, sperm counts and SOD activity compared to the I/R group. Histological evaluation confirmed the biochemical changes.
Conclusion: It is concluded that pre-treatment with SL extract protects the testis from the ischemia induced injuries.
Keywords: Testis, Stachys lavandulifolia, Ischemia

P-41: The Effect of Troxerutin Administration dc Cells in Type I Diabetic Rats

Zavvari Oskuye Z[1*], Keyhanmanesh R[1], Mirzaei F[1], Hamidian Gh[2], Mehri K[1], Ghadiri A[1]

1. Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
Email: Zohre.zavari@gmail.com

Background: In recent decades, global prevalence of diabetes has increased markedly. It is one of the most important metabolic disorders and can gradually cause damage to the function and structure of many organs such as the male gonads. Troxerutin or vitamin P4 is a flavonoid that can be found in tea, coffee and cereal and has many pharmacological properties such as anti-inflammatory and antioxidant activities but there is no data about its supportive effects on spermatogenesis process in diabetic peoples.
Materials and Methods: Fifty prepubertal (8 weeks old) male wistar rats were divided into five groups including: Control, Diabetic (DM), TX, DM+Insulin and DM+TX. Type I diabetes was induced by a single dose of streptozotocin (intraperitoneally, 55 mg/kg). Treatment groups received troxerutin (oral gavage, 150 mg/kg/day) and insulin as the reference drug (subcutaneous, 4-6 IU/day) for 4 consecutive weeks. Finally, animals euthanized and then left testes were fixed in buffered formalin 10%. The samples were processed by standard paraffin embedding and serially sectioned at 20 μm thickness. Twenty to twenty-five sections per animal were selected through systematic random sampling and stained by HandE. Total numbers of spermatogenic cells including spermatogonia, spermatocytes and spermatids were estimated by optical dissector and stereo-investigator system using an unbiased counting frame.
Results: Stereological studies showed that diabetes decreased total number of all spermatogenic cells significantly ($P<0.01$) and administration of TX could significantly inhibit this reduction similar to insulin therapy for control of hyperglycemic condition in diabetic rats ($P<0.05$). TX had no side effect on spermatogenic cell population in control animals. There is no significant difference between insulin and TX treated diabetic animals.
Conclusion: Based on our results, it can be concluded that administration of TX is a suitable protective strategy for side effect of diabetes in testis of pubertal diabetic males.
Keywords: Troxerutin, P4, Spermatogenic Cells, Pubertal, Diabetes

P-42: The Effect of Resveratrol on CatSper 1 and 2 Gene Expression in Experimental Varicocele Rat Model

Zohorsoleimani M[1*], Jalali Mashayekhi F[2], Baazm M[1]

1. Anatomy Department, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran
2. Biochemistry and Genetics, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran
Email: dr.baazm@arakmu.ac.ir

Background: Varicocele is one of the common causes of male infertility. Varicocele is an abnormal dilation in the testicular vein and caused abnormal sperm parameters such as motility. A recently characterized CatSper genes, encodes Ca channels in the testes, where they play essential roles in sperm motility. Due to the important role of antioxidant in decreasing the testis tissue damge, the aim of this research is to evaluate protective effect of resveratrol (RES) in the expression of CatSper genes, sperm parameters and testis histology following varicocele induction in rats.
Materials and Methods: In this study, 48 male Wistar rats were randomly divided into 7 groups (6 rats in each group): Control, sham, left experimental varicocele (LEV), LEV + ethanol (as vehicle), LEV + 1 mg/ kg RES, LEV + 10 mg/ kg RES, normal + 1 mg/ kg RES, normal + 10 mg/ kg RES. Varicocele was induced by partial ligation of the left renal vein.Two months after varicocele induction, resveratrol was intraperitoneally administered to rats for 2 month. Sperm parameters (count, motility, morphology and viability), testis histology (number of seminiferous and spermatogonia) and expression levels of CatSper 1 and 2 were analyzed.
Results: Our results showed that resveratrol at both doses significantly ($P\leq0.05$) increased sperm parameters, number of spermatogonia and the gene expression levels of CatSper 1 and 2.
Conclusion: Resveratrol by improving sperm parameters and the genes involved in sperm motility can be used for adjuvint therapy to reduce varicocele complication.
Keywords: Varicocele, CatSper Gene Expression, Resveratrol, Sperm Parameters

Animal Biotechnology

P-43: Effect Antioxidant The Carob Seed Extract on Sperm Motility Farahani Ram Breed after Freezing

Asgari M[*], Khodaei M, Mirzaei M, Kazemi Bonchenari M

Department of Animal Science, Agriculture and Natural Resource, Arak University, Arak, Iran
Email: mohsen.asgari20.ma@gmail.com

Background: Sperm cells have a mechanism enzymatic and non enzymatic antioxidant is but in the process Internal dilution ratio dropped antioxidants and for the treatment of sperm need to add antioxidants to foreign origin. Antioxidants are compounds the synthesis of free radicals, especially ROS control, neutral, stopped or activities to meet them. Because of safety problems, toxic substances and carcinogens In some synthetic antioxidants beta hydroxy toluene, hydroxy nyzvl, propyl gallate, etc. Economical use of natural antioxidants is taken into consideration. The antioxidant properties of plants Largely on

compounds phenolics, flavonoids, acids phenolic And phenolic diterpene to related. Objective The purpose of this study determine the antioxidant effect The carob seed extract on sperm motility farahani rams after freezing. Carob or ceratonia siliqua L. is a beautiful tree belonging to legominasae family and is about 7 to 12 meters tall. It has compound leaves and its red, yellow or purple flowers has no petal. Its arch pod fruit is bright brown with, 10 to 30 cm long and contain 12 to 16 hard seeds. Carob is native to mediterranean regions and is found in south of syria, India and most of mediterranean areas as well as in california. It grows wildly in shapoor, Fars, Iran. Studies on the chemical content carob seed it shows that contains a lot of fibers, polyphenolic compounds, arachidonic acid, lignin, fat, protein, carbohydrates, calcium, potassium and phosphorus. The carob seeds as a potential source natural antioxidant to be considered. antioxidant activity carob regarding phenolic compounds. The data revealed that the phenolic compounds of the carob powder consisted of 11 compounds. Phrogallol, catechol, chlorogenic, and protocatechuic recorded the highest values, while coumarin, cinnamic, ferulic, gallic acid, and vanillic recorded the least values of the phenolic compounds.
Materials and Methods: In this study, five farahani rams was used two to three years And semen was performed using an artificial vagina. Sperm samples after moving mixed together and sperm eighty percent of the mobility were used experiment. ejaculates were collected and diluted at a ration 1:20 with 37 ˚C extender 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). After dilution of sperm zero concentration as a witness and treatments 0.05 ml and 0.1 ml and 0.15 ml and 0.2 ml of extract carob the diluent containing the sperm was added.
Results: Results The achieved results showed that extract carob of 0/05 and 0/1 had a significantly effect on sperm protection ability and motility. Quality frozen-thawed semen of farahani ram from the significant statistical difference ($P<0.05$) was observed frozen-thawed semen quality. In frozen-thawed sperm motility was significant.
Conclusion: This treatment had a significant effect on motility sperms of farahani ram breed.
Keywords: Carob, Extract, Antioxdant, Motility Sperm, Farahani Ram Breed

P-44: *In Vitro* Effect of Progesterone on Sperm DNA Structure in Broiler Breeder Roosters

Haddad SF*, Zamiri MJ, Akhlaghi A, Jafarzade Shirazi MR

Department of Animal Science, College of Agriculture, Shiraz University, Shiraz, Iran
Email: hadad.fahime@gmail.com

Background: Progesterone (P4) is known to affect several pre-fertilization phenomena in sperm, mainly sperm motility, acrosome reaction, capacitation and intracellular calcium oscillation. Chicken seminal plasma contains high concentrations of steroid, amongst which P4 is found in higher levels. The effect of this high level of P4 on chicken sperm is not clear. However, it has been shown that addition of P4 to the rooster semen resulted in a lower rate of sperm penetration to the ovum and lower fertility. Intramuscular injection of P4 to roosters also decreased sperm motility, hypo-osmotic swelling test (HOST) and fertility duration. Sperm DNA integrity and normal chromatin are important fertility factors and there are negative correlation between DNA damage and fertility, but the effect of P4 on these parameters is not known. This experiment was designed to determine the effect of exogenous P4 on DNA structure, membrane integrity in the rooster sperm.
Materials and Methods: Eighteen Semen samples were collected from Eighteen Cobb-500 roosters, and P4 concentration in the seminal plasma was determined. Semen samples were pooled and diluted in freshly prepared Sexton extender. The extender contained 0, 2.5, 5, 10, 15, 30 ng P4 per milliliter (6 treatment groups). Diluted semen samples were evaluated at 10, 20, 40, 80 and 160 min after incubation at 39° C for sperm motility, viability (eosin-nigrosin staining), HOST, and DNA integrity by sperm chromatin structure assay (SCSA). Data were analyzed using the Proc. MIXED (SAS software). Mean comparisons were performed using the least squares means procedure adjusted for Tukey.
Results: The main effect of P4 on all sperm characteristics were significant ($P \leq 0.05$). Addition of P4 resulted in increased sperm motility, and decreased live sperm, HOST, and DNA integrity. The interaction effect of P4 × storage time was significant; 2.5 and 15ng P4/mL resulted in the highest sperm motility and the least DNA damage up to 160 min incubation. In the control semen samples, all sperm were dead after 160 min.
Conclusion: It may be that intracellular necrotic factors resulted in increased DNA fragmentation in hyper activated sperm. Increased number of such sperm in the sperm storage tubules may decrease the fertility rate of the eggs inseminated by these sperm.
Keywords: Rooster, Sperm Chromatin Structure Assay (SCSA), Sperm Motility, HOST

P-45: The Effect of Folic Acid Deprivation on Epigenetic Characteristic of Fibroblast Cells

Jozi M*, Jafarpour F, Nasr Esfahani MH

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh_nasr@med.mui.ac.ir

Background: It is well established that somatic cell nuclear transfer (SCNT) derived embryos have abnormal epigenetic reprogramming which hamper their embryonic development. This abnormal epigenetic reprogramming has been mainly related to DNA and histone hypermethylation in SCNT derived blastocysts. One of the well-known trend for improving epigenetic reprogramming in SCNT embryos is treatment of donor cells and/or reconstructed embryos with various epigenetic modifiers such as DNA methyltransferase (DNMT) inhibitors, histone deacetylase inhibitors and histone methyl transferase inhibitors. In cells, folic acid is reduced by a series of enzymes leading to the generation of tetrahydrofolate which provide methyl group for production of s-adenosyl methionine (SAM). SAM is a universal methyl donor which provides methylation for many enzymes such as DNMTs.
Materials and Methods: In this study, we designed to reduce the level of folic acid in culture medium of fibroblast cells in order to reduce the level of DNA methylation in these cells. Cells were cultured in DMEM/F-12, RPMI with folic acid (RPMI+) and RPMI without folic acid (RPMI-) medium for 6 days in presence of 0.5% FBS. The effect of folic acid deprivation were assessed on cell viability, cell cycle and global level of DNA methylation.
Results: The cell viability of cultured fibroblast cells were

100, 91.58 ± 12 and 93.76 ± 14.11 for DMEM/F-12, RPMI+ and RPMI-, respectively, which revealed that absence of folic acid didn't affect the cell viability (P>0.05). The Distribution of cells at different stages of cell cycle was similar in various treatment groups (P>0.05). Finally we assessed the global level of DNA methylation by 5-methylcytosine (5-mC) antibody using flow cytometry. Data have shown that deprivation of folic acid for 6 days reduced the intensity of 5-mC in RPMI- group (71.04 ± 8.2 %) in compared to RPMI+ group (100) (P<0.05).
Conclusion: These data may have some implications for treating of somatic donor cells in SCNT procedure for enhancing epigenetic reprogramming instead of using chemical compounds.
Keywords: Epigenetic, Folic Acid, Reprogramming, SCNT

Embryology

P-46: Ovarian Follicular Recruit Loss after Cyclophosphamide Treatment: Premature Ovarian Failure Modeling in Mice

Abedi F[1*], Abtahi NS[1], Eivazkhani F[1], Eimani H[1], Montazeri L[2], Bahrebar Kh[2], Esfandiari F[2], Fathi R[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: Rfathi79@royaninstitute.org

Background: Ovary is one of the endocrine organs of female reproductive system that produce necessary steroids and peptide hormones for puberty and the menstrual cycle. Chemo/radiotherapy lead to premature ovarian failure (POF) due to reduce follicles, ovulation and fertility. Cyclophosphamide used in the treatment of cancer is one of the usual chemotherapy and alkylating agent which cause DNA break in different type of the cells. This study focused on cyclophosphamide treatment effects on mouse ovary to produce POF model for the next studies on fertility preservation.
Materials and Methods: NMRI female mice were treated with daily intraperitoneal injection of 75 mg/kg (group A) and 100 mg/kg (group B) cyclophosphamide and kept in separate cages specific for each group for 14 days. Six-eight weeks old mice considered as control. After treatment both ovaries were removed for histological analyses. The body weight of mice were evaluated before and after injection.
Results: Histological analyses showed cyclophosphamide caused severe loss in ovarian reserve in different stages of follicles especially primordial type and injured the ovarian stromal components. Alterations of body weight demonstrated notable decrease in groups A and B as compared to control one (P<0.001). Also the results of follicular count revealed a remarkable reduction in the number of primordial follicles in the same groups compared to non-treated control group (P<0.0001). In addition, reduction of primary and preantral follicles was significant in group B against groups A and control (P<0.001 and P<0.01, resp.). Furthermore, corpus luteum formation was declined near the zero in group B (100 mg/kg) which is a robust follicular attrition sign for destruction of folliculogenesis and approving of mouse POF model.
Conclusion: According to these results, treatment of 100 mg/kg cyclophosphamide for 14 days cause a powerful damage on ovarian activity and reduce follicular reservation as compared to 75 mg/kg drug. Also lack of corpus luteum in group B showed folliculogenesis disrupt and approved creation of mouse premature ovarian failure model.
Keywords: Cyclophosphamide, Premature Ovarian Failure, Chemotherapy

P-47: Titanium Dioxide Nanoparticle Induces Apoptosis in Mouse Blastocysts

Alaee S[1*], Khodabandeh Z[2], Ilani M[3]

1. Department of Reproductive Biology, School of Advanced Medical Sciences and Technologies, Shiraz, Iran
2. Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Physiology, International Division, Shiraz University, Shiraz, Iran
Email: alaee@sums.ac.ir

Background: Titanium dioxide (TiO2) nanoparticles are widely used in many industrial products especially in skin care products and sunblocks creams. It is determined that TiO2 nanoparticles can be toxic for male and female reproductive system. In the current study we evaluated the effect of TiO2 nanoparticles on the expression of apoptotic genes in mouse blastocyst.
Materials and Methods: Female NMRI mice were divided into 3 groups. The groups consisted of a control group and experimental groups A and B, which received TiO2 (50, 100 mg/kg/day) by intraperitoneal injection for 5 consecutive days. After the last injection, pregnant mare serum gonadotropin and 48 hours later human chorionic gonadotropin were administered intraperitoneally for induction of ovulation. After 14 hours all the mice were sacrificed, cumulus-oocyte complexes were collected and invitro fertilization was carried out. After that the expression of BAX, BCL-XL and caspase 3 genes related to apoptosis was evaluated in blastocysts of each group by Real-time PCR and delta-delta CT (2-ΔΔCT) method was applied for comparing mRNA levels of activated versus control gene.
Results: The expression of BAX and caspase 3 genes as pro-apoptotic genes was significantly higher in experimental groups compared to control group. The expression of Bcl-xl gene as an anti-apoptotic gene was significantly lower in high dose group compared to control group.
Conclusion: Short-term administration of TiO2 nanoparticle in mice induces apoptosis in Blastocysts.
Keywords: Titanium Dioxide, Nanoparticle, *In Vitro* Fertilization, Embryo, Apoptosis

P-48: The Role of Zona Pellucida in Prediction of Success Assisted Reproductive Technology

Allahveisi A[1*], Nikkhoo B[2], Rezaie MJ[1], yousefian E[3]

1. Department of Pathology, Faculty Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Obstetrics and Gynecology, Faculty Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Infertility Treatment Center of Besat Hospital, Faculty Medicine, Kurdistan University Of Medical Sciences, Sanandaj, Iran
Email: allavaisie@gmail.com

Background: Poor oocyte quality may be the cause of women

infertility. Normal morphology of a healthy oocyte of metaphase II include a round even shape, light-coloured cytoplasm with homogenous granularity, a small perivitelline space without debris, and a transparent zona pellucida (ZP).In human, dysmorphology zona pellucida has an incidence of 2-5% of all oocytes.therefore Dysmorphic zona pellucida (DZP) such as ZP darkening, focal thickening, bilayering, irregular shape, debris within the zona and periviteline space may benefit from ICSI. Little studies were performed on results of the clinical of DZP. The aim of this study was to determine the role of ZP in prediction of success assisted reproductive technology (ART).
Materials and Methods: This study was performed in 97 infertile patient women with fallopian tube obstruction and/or male factor infertility which referred to Infertility Center treatment of besat hospital. All denuded oocytes which have zona pellucida subdivided into two groups: group A oocytes surrounded by normal ZP and group B surrounded by DZP. ICSI was applied in all oocytes .The fertilization, embryo quality, and the clinical pregnancy rates were evaluated in both groups.
Results: DZP of oocytes was significantly lower in other abnormality features such as dark cytoplasm with granularity cytoplasm, abnormal PB , and cytoplasmic vacuoles, refractile bodies $P<0.05$. The fertilization rate for dysmorphic zona pellucida did not differ significantly from that in normal zona pellucida $P>0.05$;Whereas the embryo quality and clinical pregnancy rates for dysmorphic zona pellucida were significantly lower than those in normal zona pellucida $P<0.05$.
Conclusion: The current study showed a correlation between dysmorphic zona pellucida of oocytes and poor embryo quality as well decreased clinical pregnancy rate. This result suggests further research to insight into success of ART.
Keywords: Dysmorphism Zona Pellucida, Fertilization, Embryo Quality, Clinical Peregnancy,

P-49: Aquaporin 3 Expression and The Role of Aquaporins in Plasma Membrane and Acrosome Integrity of Human Sperm

Alyasin A*, Momeni HR, Mahdiyeh M, Eskandari N

Department of Biology, Arak University of Science, Arak University, Arak, Iran
Email: ati.alyasin@gmail.com

Background: Aquaporins (AQPs) or water selective channels are members of the major intrinsic protein family which increase water permeability of cell membrane. AQP isoforms are present in human sperm with different localization. Several studies demonstrated the important role of AQPs as cell volume regulator where sperm experience a physiological osmotic decrease in female reproductive system. The integrity of plasma membrane and acrosome are critical parameters for sperm health. Since AQPs expression and function are crucial for normal sperm function and male fertility this study was therefore performed to investigate the expression of AQP3 and the role of AQPs in human sperm parameters such as plasma membrane and acrosome integrity.
Materials and Methods: Human ejaculated spermatozoa were washed by human tubal fluid (HTF) containing bovine serum albumin (BSA) medium. Immunocytochemistry study was performed to detect the immunolocalization of AQP3 in human sperm using a primary antibody specific for AQP3. The sperm suspension was then divided into 4 groups (each group containing 2×10^7 spermatozoa: 1. sperm at 0 hour, 2. sperm at 60 minutes (control), 3. sperm treated with mercury chloride ($HgCl_2$, AQPs inhibitor, 100 µM) for 60 minutes and 4. sperm treatment with $HgCl_2$ + mercaptoethanol (ME, 100 µM, which can partially revers the effect of $HgCl_2$). ME was used 15 minutes after $HgCl_2$ treatment. Hoechst and propidium iodide (PI) and pesium satium staining were used to assess plasma membrane and acrosome integrity respectively. Data were analyzed with one way ANOVA followed with Tukey's test.
Results: Immunocytochemistry study showed an intense AQP 3 immunoreactivity in sperm tail and a poor immunoreactivity in the acrosome. Our results also showed that plasma membrane and acrosome integrity was significantly decreased in $HgCl_2$ group. In $HgCl_2$ and ME group, ME could revers the effect of $HgCl_2$ on these sperm parameters.
Conclusion: The result of this study showed the expression of AQP 3 in the tail and acrosome of human sperm and that AQPs play role in the integrity of plasma membrane and acrosome.
Keywords: Aquaporin, Plasma Membrane Integrity, Immunocytochemistry, Human Sperm

P-50: The Effect of Cyclophosphamide on Testes Histomorphology in Offespring from Pregnant Mice Under Exposure to Cyclophosphamide

Amiri N*, Azadbakht M, Moradi N

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
Email: azadbakhtm_tmu@yahoo.com

Background: Cyclophosphamide is an alkalyn agent and one of the most common medicine that used for chemotherapy during the second and third trimester. The useing of cyclophsphamide in thses period is not safe for mother and fetus and create some abnormalities during the fetus development such as celeft palat, no eye, no finger and bone abnormalities. In additin this medicine can effect on testes development so we studied the effect of cyclophosphamide on testes histomorphology.
Materials and Methods: For this aim adult female NMRI mice were coupled with adult male mice in optimal situation and after observe vaginal plug 0 day of pregnancy fixed and divided them to two group, group 1: control group that recived salin and group 2: cyclophosphamide group that recived cyclophosphamide 10 mg/kg at 11 days of pregnancy(i.p). Male mice were sacrificed 60 days after birth and their testes removed the for tissue process.
Results: Our results showed that cyclophosphamide reduced the testes diameter, seminiferous tubule and epithelium thickness and increased the lumen dimater compared to control group. Cyclophosphamide reduced the number of sertoli cells, primery spermatocyte, round and elongated spermatide compare to control group
Conclusion: Cyclophosphamide has negative effect on testes development during the pregnancy.
Keywords: Cyclophosphamide, Testes, Chemotherapy, Pregnant Mice

P-51: The Effect of Vanadium on Testes Development in Offespring from Pregnant Mice Under Exposure to Cyclophosphamide

Amiri N*, Azadbakht M

Department of Biology, Faculty of Science, Razi University, Ker-

manshah, Iran
Email: amiri_Niloufar@yahoo.com

Background: Chemotherapy during pregnancy is one of the most important issues in these years. Cyclophosphamide is an important drug which used in the second and third trimester of pregnancy. The use of this drug can create a lot of anomalies in embryo development and reproductive organs due to cross the placenta. In addition to chemotherapy drugs, trace elements can also be used as anti-cancer drugs if their toxicity will be control. Vanadium is a trace element with anti-cancer properties which have been considered for the treatment of this disease. Low doses of vanadium do not create any problems in fetal health so perhaps this trace element with cyclophosphamide can decrease negative effects of cyclophosphamide on testes development.
Materials and Methods: For this aim we divided the female mice aged 8 weeks after pregnancy to four groups, including of control group (recived saline), cyclophosphamide group (10 mg /kg, i.p, 11 days of pregnancy), vanadium group (4 mg/kg, i.p, 8, 10, 12 days of pregnancy) and cyclophosphamide – vanadium group (10 mg /kg, i.p, 11 days of pregnancy and 4 mg/kg, i.p, 8, 10, 12 days of pregnancy). Male mice were sacrificed 60 days after birth and their testes and caudal epididym were removed for histological and sperm parameters evaluation.
Results: Sperm parameters analyzes showed that cyclphosphamide group had significant reduction of sperms count, motility, viability and significant increase in sperm abnormality compared with control group. Histological study showed the significant reduction in diameter of seminiferous tubules, germinal epithelium thickness, primary spermatocyte, sertoli cells, round and elangated spermatide in cyclophosphamide group compared to control group .However all of the adverse effects in sperm and histology parameters improved in cyclophosphamide group after exposure to vanadium.
Conclusion: Our results demonstrate that vanadium can reduce adverse effect of cyclophosphamide on male offspring during pregnancy and has protective effect on organs development against the cyclophosphamide negative effects.
Keywords: Cyclophosphamide, Vanadium, Testes, Sperm, Pregnant Mice

P-52: Royal Jelly Improves Blastulation Rate in Nicotine-Exposed Mice

Azad F[1*], Najafi Gh[2], Najati V[1], Shalizar Jalali A[2], Rahmani F[1]

1. Department of Biology, Faculty of Basic Sciences, Urmia university, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: farnam.azad@hotmail.com

Background: Nicotine is responsible for many harmful effects of cigarette smoking on reproductive health. The present study aimed to evaluate the protective effects of royal jelly (RJ) against nicotine-induced embryotoxicity in mice.
Materials and Methods: 36 male BALB/c mice were randomly categorized into six groups (n=6). Group 1 received 0.20 ml normal saline (control), group 2 received 100 mg/kg RJ, group 3 and 4 received 0.50 mg/kg and 1.00 mg/kg nicotine, respectively, group 5 received 0.50 mg/kg nicotine with 100 mg/kg RJ and group 6 received 1.00 mg/kg nicotine along with 100 mg/kg RJ. All administrations were done orally. After 30 days, blastulation rate was recorded following *in vitro* fertilization.
Results: The data indicated that nicotine significantly decreased blastulation rate ($P<0.05$) in a dose-dependent manner compared to control and RJ groups. Royal jelly co-administration improved nicotine-induced embryotoxicity.
Conclusion: Our data suggest that nicotine-induced developmental toxicities can be ameliorated by RJ co-treatment.
Keywords: Nicotine, Royal Jelly, Blastocyst, Mice

P-53: Establishment of Chemotherapy Induced Premature Ovarian Failure Model

Bahrebar Kh[1*], Esfandiari F[1], Baharvand H[1], Fathi R[2], Rezazade Valojerdi M[2, 3]

1. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department od Anatomy, Medical Science, Tarbiat Modares, Tehran, Iran
Emails: Mr_valojerdi@yahoo.com, baharvand50@yahoo.com

Background: Premature ovarian failure (POF) is an ovarian defect which is characterized by elevated and decreased levels of follicle stimulating hormone (FSH) and estrogen (E2) respectively. This conditioncauses premature ovarian follicle depletion before 40 years of age. Young women with POF suffer from infertility which is the main consequence of the disease. Moreover, chemotherapy in women with cancer usually lead to POF.Deep understanding of POF would enable us to develop advanced and effective therapy for the disease. Therefore, in this study we aimed toset up an animal model of POF that recapitulate the characteristics of the disease
Materials and Methods: In this study we used adult female C57/BL6 mice. The mice were randomly divided into four treatment groups and one control group. Female mice were intraperitoneally injected with different doses of cyclophosphamide (CTX) and busulfincluded of 50 mg/kg busulfan and 100mg/kg CTX(group 1), 100mg/kg CTX (group 2) for 10 consecutive days, 200mg/kg CTXand 50 mg/kg busulfan on the first day and then 50 mg/kg CTXand 5 mg/kg busulfan for 9consecutive days (group3) and 20 mg/kgbusulfan and 200 mg/kg CTX(group 4) a single injection. In order to evaluate whether the mouse POF model was established successfully, the body weight, estrous cyclicity, concentration of gonadal hormones, ovarian weight, and histopathology were analyzed 10 days after treatment. Ovarian specimens were collected 10 days after treatment andthe number of folliclesin all stages of maturation(primordial, primary, secondary, and antral follicles) were analyzedbyHandE staining followed by quantification of each stage. The plasma levels of E2 and FSH were measured using ELISA.
Results: The ovaries of the mice in the control group were red in color whereas the ovaries of surviving mice in the three treatments groups (named 1, 2 and 3)had pale appearance and their size were smaller than those of control mice. Mice in Groups 1, 2 and 3 after 10 days of treatment experienced estrous cycle disorders with persistent diestrus.The weight of the ovaries in the groups1, 2 and 3 mice were significantly reduced compared to control.All mice in Groups 3 and 4 survived during the observation period, whereas those in Group 1 and 2 showed a mortality rate. In addition, ovarian pathology revealed that in the groups1, 2 and 3, a significant decrease innumber of folliclesin

all stages of development (primordial , primary, secondary, and antral follicles) was detected, compared with the control group. whereas, no significant change was observed in the number of follicles in the group 4.Furthermore, the plasma E2 and levels FSH decreased and increased in the groups1, 2 and 3 micerespectively, revealsthat the POF mouse models lacked hormonal maintenance from the ovaries.
Conclusion: The results revealed that administration of chemotherapydrugscould severely disturb hormone secretion and folliculogenesis in mice.Therefore, population of follicles at different stages andovulation significantly decreased after treatment. Together our results demonstrate that group 1 and 2 mimics the POF condition and can serve as a suitable model for studying the disease.
Keywords: Premature Ovarian Failure, Folliculogenesis, Chemotherapy Drugs, Animal Model

P-54: Effect of Ellagic Acid on Total Antioxidant Capacity and Antioxidants of Ram Sperm Enzymatic

Bolouki Z*, Daghighkia H, Vaseghi Dodaran H, Najafi A

Department of Animal Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
Email: daghighkia@tabrizu.ac.ir

Background: Lipid peroxidation of the sperm membrane leads to loss of membrane fluidity and cell activity, as well as infertility. Therefore, additives with antioxidant properties should be used to reduce the effects of the cold shock of sperm cryopreservation. Superoxide anion and hydrogen peroxide are the major reactive oxygen species (ROS) in sperm cell production. The main antioxidants found in sperm cells defending against ROS are Glutathione peroxidase activity (GPx), Superoxide dismutase activity (SOD), and Total antioxidant capacity (TAC). Studies demonstrated that Ellagic acid is an antioxidant with phenolic compounds. There is a large amount of these substances in fruits, such as pomegranate, raspberry, and strawberry. The aim of this study was to investigate the effect of Ellagic acid on freezing-thawing of ram semen.
Materials and Methods: Semen samples were collected from five adult rams and diluted with a lecithin-based semen extender containing Ellagic acid (0.25, 0.5, 1, and 1.5 mM) and a control group. After freezing-thawing, total antioxidant capacity, superoxide dismutase, and glutathione peroxidase parameters were evaluated.
Results: After the thawing process, no significant differences were observed in superoxide dismutase activity and glutathione peroxidase in any of the treatment groups compared to the control group. Total antioxidant capacity increased in the intervention groups compared to the control group ($P<0.05$).
Conclusion: Our study improved sperm quality using 0.25, 0.5, 1, and 1.5 mM of Ellagic acid.
Keywords: Ram sperm, Ellagic acid, TAC, SOD, GPx

P-55: The Improvement of Ovarian Tissue Damaged by Atrazine with Aqueous Extract and Essence of Achillea Millefolium in Rat

Esmaeily Maleki R[1], Bonyadi F[2]*, Hasanzadeh Sh[2]

1. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: farzane.bonyadi@yahoo.com

Background: Using chemicals in agriculture increases production efficiency, However irreparable damage to the natural cycle of life is entered. Exposure of various pesticides has adverse effects on human health, including the pesticide atrazine that causes certain biological effects including atresia in ovarian follicles. Essence and aqueous extract of Achillea millefolium and vitamin E are important due to their preventive effects on follicular atresia. Therefore, the effects of these substances were evaluated in ovarian tissue.
Materials and Methods: 54 adult female rats divided into 9 groups. 1st and 2nd groups were received atrazine 300 and 150 mg/mg/day respectively. 3rd and 4th groups were received atrazine 300 and 150 mg/mg/day respectively along with 150 mg/kg/day water extract of A. millefolium. 5th and 6th groups were received atrazine 300 and 150 mg/mg/day respectively along with 100 mg/kg/day essence of A. millefolium. 7th and 8th groups were received atrazine 300 and 150 mg/mg/day respectively and 150 mg/kg vitamin E (a single dose intraperitoneally). The 9th group was set as control. Microscopic sections were prepared from ovaries and were investigated. Data were statistically analyzed with SPSS software by ANOVA and Turkey's tests. The significance level $P<0.05$ was considered.
Results: Results revealed that, the severity of analytical changes in untreated groups with aqueous extract and essence of A. millefolium and vitamin E were more than those groups had been treated with these substances. The results showed highly significant differences ($P<0.001$) between treated and untreated groups. Least follicular rescue and atresia were in groups treated with aqueous extract of A. millefolium.
Conclusion: The aqueous extracts and essence of the A. millefolium and vitamin E is effective in reduction of adverse changes of ovarian follicles following atrazine exposure.
Keywords: Atrazine, Achillea Millefolium, Follicular Atresia, Rat

P-56: The Effects of Vitamin D and PMSG on Development of Follicles , Granulosa Cells Diameter and mRNA Expression of Cytochrome P450 Aromatase in Cultured Ovaries

Golkarami M[1], Faraghat S[1]*, Mohammad Eini A[2]

1. Department of Veterinary Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Veterinary Basic Science Department, Ferdowsi University, Mashhad, Iran
Email: dr.faraghat7044@yahoo.com

Background: The objective of this study was to determine the actions of different concentrations of Vitamin D alone or in association with follicle-stimulating hormone (FSH) in organ culture and evaluated follicles growth, granulosa cells diameter and mRNA expression of cytochrome P450 aromatase (CYP19A1). Ovaries were cultured for 5 days in the absence or presence of Vitamin D (0.2, 1, 5 and 10 µg/ml) alone or plus PMSG (10 ng/ml). In the medium with Vitamin D 0.2 and 1 µg/ml in combination PMSG, follicles size ($P<0.01$), granulosa cells diameter ($P<0.05$) and levels of p450 aromatase ($P<0.05$) were significantly increased compared with other treatments. While in high dose of Vitamin D (5, 10 µg/ml) alone or in combination with PMSG percentage of morphologically normal follicles decreased during 5 days of *in vitro* culture ($P<0.05$). In conclusion, 0.2 and 1 µg/ml Vitamin D plus PMSG was more efficient in stimulating follicular development and increasing

expression of the CYP19A1 genes.
Materials and Methods: Culture of mouse ovaries Mouse ovaries of 5 weeks were cultured at 37°C with 5% CO_2 in 500 µl tissue-specific medium on 32-well plates. The next day, 250 µl of fresh medium was added into each well, and half of the total medium (250 µl) was replaced with fresh medium every other day. Following day 4, the basic medium was replaced by 500 µl of fresh medium every other day. The day when ovaries were placed in culture was marked as day 0. The basic medium for fetal ovarian culture consists of Dulbecco's modified Eagle's medium (DMEM)/F12 plus a-minimal essential medium (a-MEM) (1:1) (Gibco-BRL, Carlsbad, CA, USA) with 3% (w/v) BSA, 1 mg/ml of Fetuin (Sigma, St. Louis, MO, USA), 0.23 mmol/l pyruvic acid, 100 IU/ml of penicillin G, and 100 mg/ml of streptomycin sulphate (Gibco-BRL) in the absence or presence of Vitamin D (0.2, 1, 5 and 10 µg/ml) alone or in combination with PMSG (10 ng/ml). Microscopic Study After 5 days in culture, four ovaries from each treatment were fixed in 4% paraformaldehyde for 4 h and then embedded in paraffin. Serial sections (thickness, 5 µm) were cut and stained with hematoxylin-eosine. Each nucleus of oocyte from each follicles were chosen for follicle/oocyte counts, and the average was used as the follicle/oocyte number of one ovary . To assess the progression of follicle formation, we counted follicles/oocytes (primordial, preantral, antral , graaf) in ovarian sections.For measuring the diameter of ovarian follicle in each developmental stage, 45 microscopic fields were randomly chosen in each mice. Then, using the ocular micrometer of a light microscope (Olympus EH, America Inc.), at a magnification of 40×, the diameter of each ovarian follicle, oocyte, granulosa cells, internal and externa theca cells were measured. RNA preparation and Real Time PCR (RT –PCR) Total RNA was prepared from frozen ovary extraction of RNA and reverse transcription of RNA to cDNA was performed using RNX-Plus (Cinna gen, Karaj, Iran) and 2-steps RT-PCR kit (Vivantis, UK), respectively, due to manufacturer instructions. Reverse transcriptions were performed with oligo-d (T) primer at 50 8C for Hsd17b1, and with oligo-d(T) and gene-specific primer for Cyp19 (50-GACTCTCATGAATTCTCCATACATCT- 30) at 42 8C. For PCR, the primers were 50- CTGAAGCAACAGGAGTCCT AAA TGTACA-30 (sense) and 50-GGACTAGTAATGAGGGGCCCAATTCCCAGA-30 (antisense) for Cyp19. Real time-PCR performance using SYBR Green PCR Master Mix (Amplicon) and Rotor-Gene 6000 Series software version 1.7.65. Cycling conditions for Cyp19 were denaturing at 94 8C for 3 min, followed by 27 cycles of 94 8C for 45 s, 60 8C for 30 s and 72 8C for 1 min. Preliminary experiments were performed to verify that PCR product intensity increased with amount of RNA in the reverse transcription reaction, and that PCR intensity increased with cycle number. Amplification was performed within the linear range for each primer pair, and PCR productswere visualised on agarose gels after staining with ethidium bromide. Statistical Analysis The data were analyzed using SPSS, version 16 for Windows. The diameter of follicles and granulosa cells and also expression of CYP19A1 mRNA in the control and cultured groups were compared by one-way analysis of variance (ANOVA) and Tukey's post hoc test. The differences were considered to be significant when $P<0.05$.
Results: In the first set of experiment ovaries were cultured for 5 days in Dulbecco's modified Eagle's medium (DMEM)/F12 plus a-minimal essential medium (a-MEM) (1:1) with 3% (w/v) BSA, 1 mg/ml of Fetuin, 0.23 mmol/l pyruvic acid, 100 IU/ml of penicillin G, and 100 mg/ml of streptomycin sulphate in the absence or presence of Vitamin D (0.2, 1, 5 and 10 µg/ml) alone or in combination with PMSG (10 ng/ml).
Conclusion: In conclusion, low concentrations of Vitamin D with FSH were more efficient for follicles development. In fact, the combination of these hormones stimulated follicular development and maintained follicular survival as well as increased estradiol secretion. In addition, the interaction of the two hormones (FSH and Vitamin D) positively influenced diameter of follicles and granulose cellsa Thus, we have revealed combining PMSG and low concentration of Vitamin D, can influence the development of follicles and increase mRNA expression. Further research will be required to determine if exogenous Vitamin D could be used to enhance reproductive efficiency in mice.
Keywords: Vitamin D, PMSG, organ culture, granulose cells, cytochrome P450 aromatase (CYP19A1)

P-57: The Effect of Quercus Brantii Fruit Methanolic Extract on Mean Volume and Total Number of Leydig Cells in Streptozotocin Induced Diabetic Rats

Hamidian Gh*, Asghari K, Raei P

Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
Email: hamidian@tabrizu.ac.ir

Background: Diabetes mellitus (DM) is the most common endocrine disease which associated with very subtle disorders, affects, either directly or indirectly, various functions of the reproductive system. Testicular cells have their own glucose sensing machinery that react to hormonal fluctuations and have several mechanisms to counteract hyper- and hypoglycemic events. Sertoli cells (SCs), which are the main components of blood–testis barrier, are not only responsible for the physical support of germ cells but also for lactate production that is then metabolized by the developing germ cells. Any alteration in this tied metabolic cooperation may have a dramatic consequence in male fertility potential. Fruit of Iranian oak (Quercus brantii) possess many fatty acid and pleiotropic therapeutic activities that is used widely in Iranian traditional folkloric medicine. Our previous study showed that Q. brantii had a hypoglycemic effect in diabetic rats but there is no data about effects of Q. brantii fruit on mean volume and total number of Scs in diabetic males.
Materials and Methods: Twenty adult male wistar rats were divided into 4 groups including: control, sham, diabetic and treatment. Type II diabetes was induced by high fat diet and 35 mg/kg streptozotocin in diabetic and treatment groups. One week after streptozotocin injection, sham and treatment groups received 100 mg/kg/day total methanolic extract of Q. brantii by oral gavage for 40 consecutive days. Finally, animals were euthanized and testes were fixed in 10% neutral buffered formalin. The samples were processed by standard paraffin embedding and serially sectioned at 20 µm thickness. Twenty to twenty-five sections per animal were selected through systematic random sampling and stained by HandE. Mean volume of SCs (MV) was were estimated by point sampled intercept method and its total numbers (TN) were estimated by optical dissector and stereo-investigator system using an unbiased counting frame.
Results: Results showed that administration of Q. brantii had no significant effect on MV and TN of SCs in normal rats. Diabetes decreased MV and TN of SCs significantly ($P<0.05$). Results also indicated that Q. brantii could significantly increase MV and TN of Scs as to reach to normal levels. There is no significant difference between treated and control animals ($P<0.05$).

Conclusion: It can be concluded that Q. brantii can be considered as a suitable protective strategy for improvement of diabetes side effect in testis and can have a supportive effect on Sertoli cells and increase spermatogenesis process and fertilization ability in the diabetic males.
Keywords: Quercus Brantii, Sertoli Cell, Diabetes, Streptozocin, Stereology

P-58: Evaluation of Follicular Activation during The First Six Days of Life in Mouse Pups

Jafari Atrabi M[1*], Dalman A[1], Akbarnejad V[1, 2], Fathi R[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine ACECR, Tehran, iran
2. Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: rfathi79@royaninstitute.org

Background: Chemotherapy and radiotherapy could detrimentally impact primordial ovarian follicles leading to premature ovarian failure. *In vitro* culture of follicles is considered as one of the techniques for preservation of fertility in these patients; however, the optimum *in vitro* condition has not been established yet.
Materials and Methods: In this regard, knowledge of the changes in primordial follicles and gene expression of factors contributing to follicular activation could help tailor the optimum in vitro condition for activation of follicles. Accordingly, the present study was designed to evaluate different stages of follicles as well as gene expression of phosphatase and tensin homolog (PTEN), phosphoinositide 3-kinase (PI3K) and connexin 37 (CX37) in one-day- and six-day-old mice. While merely primordial follicles were observed in the ovary of one-day-old mice, various stages including transitional, primary and preantral follicles were present in the ovary of six-day-old mice.
Results: As a result, the proportion of primordial follicles decreased over time ($P < 0.05$), whereas the proportion of transitional, primary and preantral follicles increased over the course of six-day period ($P < 0.05$). Concomitant with activation of follicles in six-day-old mice, the gene expression of PTEN and CX37 was elevated ($P < 0.05$), but PI3K gene expression remained unchanged ($P > 0.05$). Concomitant with morphologically activation of follicles in six-day-old mice, the gene expression of PTEN and CX37 was elevated ($P < 0.05$), but PI3K gene expression remained unchanged ($P > 0.05$).
Conclusion: In conclusion, the present study revealed the activation of follicles up to the preantral stage in six-day-old mice, which was accompanied with upregulation of PTEN and CX37.
Keywords: Ovarian follicle activation, CX37, PTEN,PI3K,

P-59: A Stereological Evaluation on The Effect of Bone Marrow-Derived Mesenchymal Stem Cells Transplantation on Fullicolugenesis in Mice following Induction of Polycystic Ovary Syndrome

Kalhori Z[1*], Soleimani Mehranjani M[1], Azadbakht M[2], Shariatzadeh MA[1]

1. Department of Biology, Faculty of Science, University of Kermanshah, Kermanshah, Iran
2. Department of Biology, Faculty of Science, University of Arak, Arak, Iran
Email: kalhoriz@ymail.com

Background: Polycystic ovary syndrome (PCOS) is the most endocrine disorders leading to abnormal folliculogenesis due to insulin resistance, hyperandrogenism and inflammation. Bone Mesenchymal stem cells (BMMSCs) have immunomodulation properties and their therapeutic effect is due to their immunoregulatory functions. The aim of this investigation was to evaluate the effect of BMMSCs transplantation on improving the histological indexes of fullicolugenesis in mice with induced PCOS using stereological techniques.
Materials and Methods: PCOS was induced through daily injections of testosterone enanthate (1 mg/100g s.c. for 5 weeks). NMRI mice (3 weeks old) were divided into 3 groups (n=6): Control, PCOS, PCOS +BMMSCs .BMSCs injected into the mice (106MSCs/30g body weight, via the tail vein) at 1 and 14 days after induction of PCOS, the mice were killed at 2 weeks after last transplantation. The ovaries were then studied stereologically. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at ($P<0.05$).
Results: The mean total volume of ovary, the volume of cortex and the number of antral follicles reduced significantly in the PCOS group compared to the control, while these parameters significantly increased in the PCOS+BMMSCs group compared to the PCOS group. The number of primary and preantral follicles showed a significant increase in the PCOS group when compared to the control but a significant reduction was found in the mentioned parameters in the PCOS+BMMSCs group. The volume of oocyte and the thickness of zona pellucida (ZP) in the preantral and antral follicles significantly increased in PCOS+BMMSCs group to the control level when compared to the PCOS group.
Conclusion: BMMSCs can improve the folliculogenesis through enhancing the favorable ovary histological changes in mice with induced PCOS.
Keywords: Polycystic Ovary Syndrome, Bmmscs, Stereology, Mouse

P-60: Intravenous Injection of Bone Marrow Mesenchymal Stem Cells Improves The Ovary Function through Reduction of Inflammatory Factors in Mice with Induced Polycystic Ovary Syndrome

Kalhori Z[1*], Soleimani Mehranjani M[1], Azadbakht M[2], Shariatzadeh MA[1]

1. Department of Biology, University of Arak, Arak, Iran
2. Department of Biology, University of Kermanshah, Kermanshah, Iran
Email: kalhoriz@ymail.com

Background: Polycystic ovary syndrome (PCOS) affects between 5–10% of women in reproductive age. It is believed that inflammation play an important role in the pathogenesis of PCOS. Bone marrow mesenchymal stem cells (BMMSCs) have anti-inflammatory property and repair damaged tissues by expressing the immunosuppressive molecules. The aim of present study was to investigate the anti-inflammatory effect of BMSCs on the function of ovary in mice with induced PCOS.
Materials and Methods: Mouse model of PCOS was performed through daily injection of testosterone enanthate (1 mg/100g/ body weight s.c.) for a period of 5 weeks. NMRI mice (3 weeks old) were divided into three groups (n=6): Control, PCOS and PCOS + BMMSCs. BMSCs injected into the mice (106MSCs/30g body weight, via the tail vein) at 1 and 14 days after induction of PCOS

and then followed by determining the estrous cycle one day after MSCs injection. FSH, LH, testosterone, IL-6 and TNF-α serum levels also measured using ELISA kit. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at ($P<0.05$).
Results: Estrous cycle was not recovered in the PCOS group while in the PCOS+BMMSCs group it was recovered. The serum levels of testosterone, LH, IL-6 and TNF-α were significantly higher and the serum level of FSH was significantly lower in the PCOS group when compared to the other groups. A significant reduction in the Serum levels of testosterone, LH, IL-6 and TNF-α were found in the PCOS +BMMSCs group when compared to the PCOS group. A significant increase was seen in the Serum level of FSH in PCOS+BMMSC group in comparing with PCOS ones.
Conclusion: Our results showed that BMMSCs could improve endocrine function of ovary through favorable changes in the mean concentrations of FSH, LH, testosterone, IL-6 and TNF-α serum in mice with induced PCOS.
Keywords: Polycystic Ovary Syndrome, BMMSC, Inflammation, Mouse

P-61: Clinical Evaluating The Effectiveness of Combination of Density Gradient and Zeta Methods in Isolation Mature Sperm

Karimi N[1*], Kouchesfehani H[1], Nasr Esfahani MH[3], Shahverdi AH[2], Chobineh H[4], Esmaeili V[2]

1. Animal Science, Biological Science, Kharazmi, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
4. Infertility Center of The Iranian Red Crescent, Tehran, Iran
Email: kariminazanin62@gmail.com

Background: Selection of sperm for intra-cytoplasmic sperm injection (ICSI) is usually considered as the ultimate technique to alleviate male-factor infertility. In routine ICSI, selection is based on morphology and viability which does not necessarily preclude the chance injection of DNA-damaged or apoptotic sperm into the oocyte. Sperm with high negative surface electrical charge, named "Zeta potential", are mature and more likely to have intact chromatin. Therefore, we aimed to compare the clinical outcomes of DGC/ Zeta procedure with routine sperm selection in infertile men candidate for ICSI.
Materials and Methods: From a total of 100 ICSI cycles studied, 50 cycles were allocated to density gradient centrifugation (DGC)/Zeta group and the remaining 50 were included in the DGC group in this prospective study. Clinical outcomes were com¬pared between the two groups.
Results: In the present randomized clinical trial, a significant increase in top quality embryos and pregnancy rate were observed in DGC/Zeta group compared to DGC group.
Conclusion: Zeta method may improve the percentage of top embryo quality and pregnancy outcome compared to the conventional DGC method.
Keywords: Zeta Potential, Density Gradient Centrifugation, Embryo Quality

P-62: Inhibitory Effect of Noscapine on Human Endometrial Tissue in Three-Dimensional Culture Model of Endometriosis Through Reduction of Nitric Oxide and Expression of Apoptotic Genes

Khazaei MR[1*], Nasr Esfahani M[2], Chobsaz F[1], Khazaei M[1]

1. Fertility and Infertility Research Center, Kermanshah University of Medical Science, Kermanshah, Iran
2. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mrasoolkhazaei@gmail.com

Background: Human endometrium is a highly regenerative tissue with central role in reproduction. Noscapine is an alkaloid derived from Papaver somniferum (Papaveraceae family) with anti-cancer activity. It is candidate for endometriosis treatment. The aim of present study was to examine the effect of different doses of noscapine on human endometrial tissue in a three-dimensional culture model of endometriosis.
Materials and Methods: In this *in vitro* study, normal human endometrial tissues (n=8) were taken and were divided to 5 groups. Control groups which received M199 medium containing 5% fetal bovine serum (FBS), and test groups were exposed to media containing 10, 50, 100 and 200 μM of noscapine, for 21 days. The growth score, nitric oxide (NO) level, angiogenesis, and the expression of apoptotic genes were studied. One-way ANOVA were performed to compare the groups.
Results: The mean of growth score of endometrial explants in control (0), 10, 50, 100 and 200 μM of noscapine, were 2.11 ± 0.6, 1.65 ± 0.5, 0.79 ± 0.41, 0.18 ± 0.1 and 0.1 ± 0.1 in normal endometrium ($P<0.05$).. Also, the mean of NO levels were 27.4 ± 3.42 23.19 ± 5.27, 19.24 ± 5.02, 15.77 ± 3.89 and 10.57 ± 1.65, respectively with significant difference ($P<0.01$). In endometrial tissues, The expression of p53, Bax, caspase 3, caspase 8 in normal specimens increased 5, 2.5, 2 and 2.5 folds compared to untreated control, while levels of Bcl2 and Sirt1 was determined.
Conclusion: Noscapine showed a time- and dose-dependent inhibitory effect on human endometrial tissue. The expression of apoptotic genes significantly increased while the levels of Bcl2 and Sirt1 reduced.
Keywords: Endometriosis, Noscapine, Apoptosis, Nitric oxide, Sirtuin

P-63: Effect of Addition Eggplant Peel Extract on Ram Sperm Motility after Thawing

Khodadadi H[1*], Khodaei Motlagh M[1], KazemiBonchenari M[1], Momeni H[2]

1. Animal Science, Agriculture and Natural Resource, University of Arak, Arak, Iran
2. Department of Biology, Faculty of Basic Sciene, Arak University, Arak, Iran
Email: khodadadi_h_67@yahoo.com

Background: Eggplant is one of the contains a hight amount of (ROS) oxygen radical scavenging. The eggplant (Solanum melongena) is a vegetable typical of the Mediterranean and is a rich phenolic compounds while is also a powerful antioxidant for prevention of disease. Anthocyanines is an important pigments in this plant and is a below group of phenolic compounds which soluble in water and is the main phenolic compounds in eggplant peel. Anthocyanins are the group of flavonoids which

protection from the eggplants in contrast the damages. From the best antioxidants in eggplants is nasunin which use for the free radicals oxygen battle. Nasunin identified in the peel of eggplants such as (aubergine) which has been protected with peel of plant and is a very strong antioxidant in plants. This study is to determine the effect of extract of solanum melongena (aubergine) on semen factors of farahani ram breed. The sperm motility has been considered as a fertility parameter in the present study.

Materials and Methods: In this study five farahani rams were allocated in a completely randomized design (averaging 2.5 years old). The protocol for use of animals in these experiments was approved by the Arak University in Iran. Semen was obtained from these rams. The rams had free access to feed and watrer. Semen sample of rams were collected by artificial vagina. Ability of sperms were greater than 80% motility and concentration normal. A total number of 40 semen samples (8 ejaculates from each ram) were evaluated in the study. ejaculates were collected and diluted at a ration 1:20 with 37 ˚C extender 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). The experimental concentrations of eggplant extract were: 2, 4, 6, and 8%. Eggplant extract was not used for control treatment. The rang of PH extract acidity and alkalinity conditions was 5.5 to 7.

Results: This study cleared that 2 percent of eggplant extract (aubergine peel extract) had higher than motility compared to other treatments with 62.65% ($P<0.05$). The results show that greater levels of eggplant extract would cause negative effect on sperm quality which might be related in to toxic compounds find in these extract.

Conclusion: This report show that this extract can be use for cryopreservation of sperm in long time.The results show that 2% of eggplant extract added in to ram semen could improve sperm motility; however based on the results acquired in the present study greater level addition did not granted more useful effect.

Keywords: Eggplant Peel, Extract, Farahani Ram, Motility Sperm, Antioxidant

P-64: The Study Effect of Folic Acid and GnRh on Development of Follicles, Granulosa Cells Diameter in Cultured Ovaries

Khorrami N*, Alinezhad G

Department of Veterinary, Science and Research Branch, Islamic Azad, Tehran, Iran
Email: niloo.kh.71@gmail.com

Background: This study was to determine the actions of different concentrations of folic acid alone or in association with GnRH in organ culture and evaluated follicles growth, granulosa cells diameter and mRNA expression of cytochrome P450 aromatase.

Materials and Methods: Mouse ovaries of 5 weeks were cultured at 37°C with 5% CO_2 in 500 µl tissue-specific medium on 32-well plates (21). The next day, 250 µl of fresh medium was added into each well, and half of the total medium (250 µl) was replaced with fresh medium every other day. Following day 4, basic medium was replaced by 500 µl of fresh medium every other day. Day when ovaries were placed in culture was marked as day 0. The basic medium for fetal ovarian culture consists of dulbecco's modified eagle's medium (DMEM)/F12 plus a-minimal essential medium (a-MEM) (1:1) (Gibco-BRL, Carlsbad, CA, USA) with 3% (w/v) BSA, 1 mg/ml of fetuin (Sigma, St. Louis, MO, USA), 0.23 mmol/l pyruvic acid, 100 IU/ml of penicillin G, and 100 mg/ml of streptomycin sulphate (Gibco-BRL) in the absence or presence of folic acid (0.2, 1, 5 and 10 µg/ml) alone or in combination with GNRH (10 ng/ml).

Results: In the first set of experiment ovaries were cultured for 5 days in dulbecco's modified eagle's medium (DMEM)/F12 plus a-minimal essential medium (a-MEM) (1:1) with 3% (w/v) BSA, 1 mg/ml of fetuin, 0.23 mmol/l pyruvic acid, 100 IU/ml of penicillin G, and 100 mg/ml of streptomycin sulphate in the a bsence or presence of folic acid (0.2, 1, 5 and 10 µg/ml) Alone or in combination with GNRH (10 ng/ml).

Conclusion: We have revealed combining GNRH and low concentration of folic acid, can influence the development of follicles and increase mRNA expression. The combination of folic acid with FSH, stimulated follicular development and interaction of the two hormones positively influenced diameter of follicles and granulose cells.

Keywords: Folic Acid, GNRH, Organ Culture, Granulose Cells, Cytochrome P450 Aromatase

P-65: Royal Jelly Improves Blastocyst Hatching Rate in Heat Stress Exposed Rats

Mahdivand N[1*], Najafi Gh[2], Nejati V[1], Shalizar Jalali A[2], Rahmani F[1]

1. Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: n_mehdivand@yahoo.com

Background: Scrotal hyperthermia has been known as a cause of male infertility. This study was conducted to determine the protective role of royal jelly (RJ) in blastocyst hatching rate following heat stress induction (37, 39 and 43°C for 20 min) in male rats.

Materials and Methods: 40 adult male rats were randomly divided in to the 8 groups (n=5) including control, RJ, 43°C, 39°C, 37°C, 43°C+RJ, 39°C+RJ and 37°C+RJ. Royal jelly was administered at a dose of 100 mg/kg by oral gavages. After 48 days, blastocyst hatching rate was recorded following *in vitro* fertilization.

Results: The results showed that blastocyst hatching rate decreased significantly ($P<0.05$) in heat stress group compared to control and RJ groups. Notably, RJ co-administration improved heat stress induced embryo development arrest.

Conclusion: These findings indicate that RJ can improve early embryo development following heat stress induction in rats.

Keywords: Blastocyst, Heat Stress, Royal Jelly, Rat

P-66: Effects of Royal Jelly on Heat Stress Induced Alterations in Epididymal Sperm Apoptosis and Nuclear Maturity in Male Rats

Mahdivand N[1*], Nejati V[1], Najafi Gh[2], Shalizar Jalali A[2], Rahmani F[1]

1. Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Email: n_mehdivand@yahoo.com

Background: Spermatogenesis is a temperature-dependent process and increases in scrotal temperature can disrupt its progression. We used a rat model to examine the scrotal heat stress (37, 39 and 43 °C for 20 min) effects on epididymal sperm apoptosis and nuclear maturity as well as possible repro-protective activities of royal jelly (RJ).
Materials and Methods: 40 adult male rats were randomly divided in to the 8 groups (n=5) including control, RJ, 43°C, 39°C, 37°C, 43°C+RJ, 39°C+RJ and 37°C+RJ. Royal jelly was administered at a dose of 100 mg/kg by oral gavages. After 48 days, epididymal sperm apoptosis and nuclear maturity were assessed through Annexin V and aniline blue staining, respectively.
Results: The results showed that epididymal sperm apoptosis and positive reaction to aniline blue staining significantly ($P<0.05$) increased following heat stress induction. Noticeably; RJ administration improved above-mentioned parameters.
Conclusion: These findings demonstrate that scrotal heat stress can lead to elevated apoptosis and disturbed nuclear maturity in epididymal sperm. Further, it seems that RJ treatment can decrease scrotal heat stress related epididymal sperm impairments.
Keywords: Heat Stress, Royal Jelly, Apoptosis, Sperm, Rat

P-67: Foeniculum Vulgare Effects on Testicular Tissue and Two Cell Embryo Development

Minas A[1*], Rezaei Agdam H[2], Talebi H[3], Daliri Z[4], Moshari S[5]

1. Basic Science, Faculty of Veterinary, Urmia university, Urmia, Iran
2. Private Veterinary Practitioner, Urmia, Iran
3. Department o Veterinary, Islamic Azad University, Urmia Branch, Urmia, Iran
4. Department of Biology, Islamic Azad University, Tehran Branch, Tehran, Iran
5. Department of Biology, Faculty of Basic Science, Urmia University, Urmia, Iran
Email: aram.minas.reyhanabad@gmail.com

Background: Fennel (Foeniculum vulgare), a well-known medical plant is used extensively due to its pharmacological properties. In present study we examined the Fennel effects on testicular tissue and also embryo development in animal models.
Materials and Methods: Twenty adult male mice were randomly categorized to control and test groups. The experimental group subdivided into three groups, which received 0.37 mg/kg, 0.75 mg/kg and 1.5 mg/kg of fennel essential oil orally. After 35 days, epididymal sperm were samplized and following *in vitro* fertilization after 24 hours two cell embryo were evaluated. Moreover testicles were dissected and fixed in 10% fixative solution. Fixed samples were paraffin-embedded and cut into 5µm thick and routinely stained with Haematoxylin and Eosin (HandE). Finally, histopathology examinations were done and analyzed by SPSS software.
Results: Our histopathology results revealed that, the spermatogenesis and differentiation processes were diminished in fennel-administrated groups, dose dependently. Accordingly, the animals in fennel-administrated groups exhibited arrested spermatogenesis (1.5 mg/kg-received group), impaired tubular differentiation index and spermiogenesis ratio (in 0.75 and 1.5 mg/kg-received groups). No histopathological changes were observed in control group. Additionally, *in vitro* fertilization data illustrated diminished two-cell embryo development in fennel-administrated groups.
Conclusion: Our findings showed that, the administration of fennel reduces spermatogenesis, which in turn adversely affects sperm quality and finally leads to low *in vitro* fertilization as well as embryo development.
Keywords: Fennl, Testis, Fertilization, Embryo

P-68: The Study of Protection Effect of Komboucha Against The Toxicity Induced Silver Nanoparticle on The Testis Tissue of Mice NMRI

Miri SA[*], Shariatzade SM

Department of Biology, Faculty of Science, Arak University, Arak, Iran
Email: am.miri9450@gmail.com

Background: Silver nanoparticles have frequeny application in modern technology specially medical.these particles due to their small size crosses of cell membrans and blood testis barrier and is causes oxidative stress in the male reproductive system . Kombucha is a fermental drink with detoxification property and powerful antioxidant. The aim was to investigate the protection effect of kombucha as an antioxidant against damages induced of caused of silver on the mice testis tissue.
Materials and Methods: 24 adult male mice of NMRI with weight average (30 ± 2), were devided into four groups(n=6) : control, silver nanoparticles(500 mg/kg/day), kombucha(9 ml/kg/day) , and silver nanoparticles+kombucha. treatment was performed orally and through gavage for 35day. Then testis tissue is removed and evaluated histopathologcal changes. blood serum collected and were used for evaluation of biochimecal. data were analyzed with one-way anova and means difference wase considered significant when $P<0.05$
Results: The level of testosteron hormone and total antioxidant capaity rate decreased significantly in the silver nanoparticale group.further, MDA level increased significantly in this group compared to the control group.these parameters increased in the Kombucha+ nanoparticale group compared to the nanopartical group.Histologitudy studies of treatmented mice testis with nanoparticle, showed vacuolization and reducing the thickness of the germinal epithelium and reducing the number of sperms in the lumen of the seminiferous tubules. On the other hand, in the simultaneous kombucha treatment group and silver nanoparticale. above changes improved in the control group.
Conclusion: It seems to Kombucha as an free radical inhibitor, is enable to reducing oxidative damages of testis tissue and possibly can improve adverse effects of silver nanoparticle on male reproduction system.
Keywords: Silver Nanoparticles, Kambucha, Histo Pathology, Testis, NMRI Male

P-69: Mouse Visceral Peritoneum Mesenchymal Stem Cells Express Germ and Oocyte Cells Markers during *In Vitro* Induction by Human Follicular Fluid

Mirzaeian L[1*], Esfandiari F[1], Rezazadeh Volojerdi M[2, 3], Fathi R[3]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

3. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: rfathi79@royaninstitute.org

Background: The mesothelium is a thin layer of the serous lining embryonic body cavities and surrounds internal abdominal/thoracic organs. Increasing of body evidences has confirmed existence and special functions of peritoneum mesenchymal stem cells (PMSCs) which have a same origin with ovarian surface epithelium (OSE). In the present research, PMSCs were isolated from intestinal peritoneal mesothelium and differentiated into germ and oocyte like cells with a novel approach on using of human follicular fluid.
Materials and Methods: Direct explants of mouse intestinal peritoneal mesothelium have been used as the source of mesenchymal stem cells. The cells were maintained in DMEM F12 medium supplemented with 15% fetal bovine serum (FBS). After 2-3 passages, PMSCs were morphologically evaluated then subsequently assessed for proliferation, cell markers expression and differentiation to osteocyte and adipocyte. Finally PMSCs were cultured using of 10% human follicular fluid (HFF) for 21 days *in vitro*. PMSCs were assessed with immunofluorescence for expression of PGC specific proteins, Dead (Asp-Glu-Ala-Asp) box polypeptide4 (Ddx4) and deleted in azoospermia like (Dazl) and oocyte specific markers; Growth differentiation factor-9 (Gdf9), Zona pellucida glycoprotein3 (Zp3).
Results: PMSCs, Morphologically, formed a homogeneous cell population after 3-4 passages and the representative graph for cell proliferation showed increased cell viability (MTT test). Furthermore, PMSCs could be differentiated to adipo/osteocytes and phenotypic characterization revealed the positive expression of cytokeratin19, CD44 and CD90. Finally PMSCs produced oocyte-like cells and express germ and oocyte cells specific markers.
Conclusion: This study indicated that the mesothelium contained mesenchymal stem cells which are able to differentiate into oocyte-like structures by the means of human follicular fluid.
Keywords: Peritoneum, Mesothelial Mesenchymal Stem Cells, Oocyte-Like Cells, Germ Cell Markers

P-70: The Study of Effects of Malva Sylvestris Extract in Oocyte Maturity and Fertilizing in Granolusa Cells Co-Culture in Goat

Mohammad Eini A[1], Mozafari M[2], Ahmadifar M[1*], Vahidi-Erysofla N[4], Lotfi Panah M[2]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: Mehdi_Ahmadifar67@yahoo.com

Background: The laboratory animal embryo production is developed in recent years. The improvement of production process of this embryo increase the performance of laboratory to produce this embryo and also increase the chance of success fertility after implant. The oocyte maturity and fertilizing in laboratory is a technic that can cause decrease the costs and side effect of using gonadotropin for laboratory fertilize. The improvement and development of the laboratory culture for oocytes maturity is an important progress in infertility in human and animal. Research and investigation in this context of human biology is so hard. Today usage of animal for studding in oocyte maturity provided perfect system improved laboratory environments consist of maturity culture, oocyte fertility, using stimulants maturity materials.
Materials and Methods: In this study we investigated the effect of different doses (0.5-2-5-10 Nm) of Malva in compare with negative and positive control in co-culture granolusa cells and we studied the rate of oocyte fertilization and maturity in goat.
Results: This study showed the does increasing of Malva extract (until 5nM) increased the rate of maturity and fertility, but in 10nM level decrease them. We have significant differences between treatment group (5nM) and other (negative and positive control and 10nM).
Conclusion: The co-culture between goat granolusa cells and oocytes in 5 mg/ml of Malva Sylestris can increase the performance in maturity cultures and oocytes fertilizing.
Keywords: Co-Culture in Granolusa Cells, Malva Sylvestris Extract, Fertility, Maturity, Goat Ovule

P-71: Influential Effect of Age on Oocyte Morphometry, Fertilization Rate and Embryo Development in Mice

Mohammadzadeh M[*], Moshrefi M, Khalili MA

Biology and Anatomical Sciences, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran
Email: khalili59@hotmail.com

Background: Changes of oocyte quality and decreasing of ovarian follicle reserve in advanced age are the major challenges in infertility treatments. One factor associated with ageing is the thickness of zona pellucida (ZP), which has adverse relation with embryo score. Although, there is no correlation between perivitelline space (PVS) and granulation with embryo quality, an inversely correlation is observed between these two factors and subsequent embryo quality. The aim was to investigate the influence of age on oocyte quality, fertilization rate and embryo development in mice.
Materials and Methods: NMRI mice (No=21) were categorized into 3 groups regarding to their ages (25, 30, 35 weeks old). *In vitro* fertilization (IVF) protocol was conducted for each group. After collection of the oocytes, three points of ZP and PVS diameters were measured in each group and mean values were calculated. Also, the number of oocytes, fertilization rate, the number of 2 and 4 cells embryos and blastocyst formation were compared among the groups. The results were analyzed by one way Anova fallowed by Post Hoc test.
Results: All the changes were age related. So that, the mean value of ZP and PVS diameters as well as the number of oocytes, rat of fertilization, 2 and 4 cells embryos and blastocysts were higher in the younger group (25 week) compared to two other groups. Also, no significant difference (P value≤0.05) was observed between the evaluated parameters, except for blastocyst formation rate.
Conclusion: Although, the advanced age influenced negatively on oocyte morphometry and cleavage and blastocyst embryo formation, this effect was significant only for blastocyst formation.
Keywords: *In Vitro* Fertilization, Zona Pellucida, PVS, Oocyte, Age

P-72: The Effect of Maternal Diabetes on Histomorphology

of Offspring's Testis

Moradi N*, Azadbakht M, Amiri N

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
Email: Negarmoradi1511@gmail.com

Background: Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of variable degree with onset or recognition during pregnancy. These diseases are growing due to lifestyle, industrial expansion, the prevalence of obesity and type 2 diabetes. In other side diabetes has an adverse effect on male reproductive function. The atrophic changes base on histological studies, such as decreased tubular diameter, surface, and volume density of these regions was observed.
Materials and Methods: We have used pregnant NMRI mice in 2 groups: A (control), B (diabetes). In Groups B at the third day of pregnancy, 200 mg/ kg of alloxan was injected intraperitoneally. After the birth and maturation of male mice, we have evaluated their testicular tissue.
Results: Our results showed the gestational diabetes, impaired performance and reduced male reproductive system, including significant reduction in testicular weight, reduction in testis diameter, seminiferous tube, the epithelial thickness, as well as reduced the number of Sertoli cells, early spermatocytes, round and elongated spermatides in alloxan-treated mice.
Conclusion: Maternal diabetes during pregnancy can affect the reproductive system of male mice. These findings may explain the negative reproductive consequences on children of diabetic mothers.
Keywords: Gestational Diabetes, Testes, Histomorphology, Pregnant Mice

P-73: Effect of Vanadium on Testis Development of Diabetic Mother's Offspring

Moradi N*, Azadbakht M

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
Email: Negarmoradi1511@gmail.com

Background: Any degree of glucose intolerance with the onset or first recognition during pregnancy is defined as Gestational Diabetes Mellitus (GDM). GDM occurs if pancreatic b-cells are unable to face the increased insulin demand during pregnancy. These diseases are growing due to lifestyle industrial expansion, the prevalence of obesity and type 2 diabetes. In other side diabetes has an adverse effect on male reproductive function, All of the sperm parameters of Alloxan-induced diabetic rats were significantly lower than control values. In many research studies a significant relationship was observed between diabetes mellitus and trace elements. Insulin action was reported to be potentiated by some trace elements like vanadium. Vanadium and its compounds exhibit a wide variety of insulin-like effects. In this study, these effects are discussed with respect to the treatment GDM.
Materials and Methods: We have used 20 pregnant mice in 4 groups (N=5) : A (control), B (diabetes), C (vanadium), and D (treatment). In Groups B and D on the third day of pregnancy, 200 mg/ kg of alloxan was injected intraperitoneally . After 72 hours in the 6, 8 and 10 days of pregnancy 4 mg/ kg of vanadium was injected in C and D groups . After the birth and maturation born, we tested their sperm parameters and testicular tissue.
Results: All of reproductive parameters included Sperm count, viability, motility, testis diameters, seminiferous tube diameters, epithelium thickness of seminiferous tube and also the number of sertoli cell, early spermatocyte, round and elongated spermatid were decreased in alloxan-treated mice compared to control group and improved after exposure to vanadium.
Conclusion: Maternal diabetes during pregnancy can affect the reproductive system of male born. These findings may explain the negative reproductive consequences on children of diabetic mothers and vanadium could improved these adverse effects.
Keywords: Gestational Diabetes, Testes, Pregnant Mice

P-74: The Maturation and Fertilization Rate of Co Culture Granolosa Cells and Oocytes Between ICSI and IVF

Mosallanezhad Z[1*], Ghobadifar M[2]

1. Department of Gynecology and Obstetrics, Medical Sciences, Jahrom, Iran
2. Zoonoses Research Center Faculty of Medicine, Medical Sciences, Jahrom, Jahrom, Iran
Email: zahramosallanezhad@hotmail.com

Background: To consider which methods of micromanipulation techniques increases the human oocyte maturation *in vitro* and fertilization rate cultured with or without CCs, we aimed to compare intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF).
Materials and Methods: Five hundred fifty immature oocytes were retrieved and were randomly divided into two groups; oocytes that were cultured with CCs (Group A) and oocytes cultured without CCs (Group B). After *in vitro* maturation (IVM), only oocytes that displayed Metaphase II (M II) stage went randomly through the ICSI or IVF procedure. Maturation and fertilization rates were all examined.
Results: The mean age, basal follicle-stimulating hormone (FSH), and number of oocytes recovered for the patients were all comparable between the two study groups. The number of oocytes that reached M II (mature oocytes) was 194 out of 275 in group A(CC-co cultured group) compared to 165/275 in group B (P=0.009). The fertilization rates of matured human oocytes cultured with and without CCs by ICSI procedure was significantly higher than IVF method (P=0.005 vs. P=0.001, respectively).
Conclusion: Findings of the current study revealed that the fertilization rate of *in vitro* matured oocytes during ICSI procedure is higher than IVF method.
Keywords: Fertilization, Immature Oocytes, Intracytoplasmic Sperm Injection, *In Vitro* Fertilization, Cumulus Cells

P-75: Aflatoxin B1-Induced Atresia in Ovarian Tissue Correlates with Oxidative Stress and Consequent Mitochondria-Dependent Apoptosis

Poursadeghi F[2], Razi M[1*], Amniattalab A[2]

1. Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Pathology, Faculty of Veterinary Medicine, Islamic Azad University, Urmia Branch, Urmia, Iran
Email: mazdak.razi@gmail.com

Background: Dietary toxins such as different mycotoxins

are known as major contributors resulting in male reproductive health failure. Among various mycotoxins, including zearalenone, ochratoxins, fumonisins and tremorgeic toxins, the aflatoxins (AFs) are mostly diagnosed in food and feed commodities. Among all subtypes of AFs, the AFB1 is known as most harmful AF, which is able to adversely affect female reproductive potential. The intrinsic apoptosis pathway is mainly based on impaired mitochondrial membrane transition and cytochrome c release into cytoplasm. Considering AFB1-induced detrimental effect on different biological systems, present study was performed to assay mitochondria-dependent apoptosis following exposure to AFB1 at different time periods. For this purpose, possible changes in expression of Bcl-2, p53, Bax and caspase-3 genes and/or proteins were evaluated.
Materials and Methods: To follow-up present study, 24 mature female Swiss albino mice were randomly divided into control and test groups. The animals in test group subdivided into three groups, which received the AFB1 at a daily dose of 20 µg/kg body weight, through intraperitoneal (i.p.) route, for 7, 14 and 21 days. The expression of Bcl-2, Bax, p53 and caspase-3 at both mRNA and protein levels were analyzed by using reverse transcription PCR (RT-PCR) and immunohistochemistry, respectively. The atretic follicles number/one ovary was evaluated. Moreover, the apoptosis was assessed by using TUNEL staining. Finally, ovarian total antioxidant capacity (TAC) and malondialdehyde (MDA) contents were evaluated and compared between groups.
Results: Observations showed that, the AFB1 increased follicular atresia in a time dependent manner. The AFB1-received animals exhibited increased ($P<0.05$) expression of Bax, p53 and casapsae-3 expression at both mRNA and protein levels. However, the expression of Bcl-2 was decreased time dependently versus control group. The AFB1 significantly ($P<0.05$) enhanced the apoptosis index and remarkably ($P<0.05$) diminished ovarian antioxidant status. Accordingly, the animals in AFB1-received group exhibited decreased TAC level and increased MDA content versus control group.
Conclusion: Our data showed that, the AFB1-induce oxidative stress in association with diminished Bcl-2 expression results in remarkable Bax oligomerization and consequent cytochrome c release into cytoplasm. In line with these issues, the AFB1 triggers caspase-3 expression (as finisher protein), which in turn results in massive apoptosis at oocyte and follicular cells level. On the other hand, severe DNA fragmentation initiates the p53 overexpression, which in turn accelerates the apoptosis process.
Keywords: Aflatoxin B1, Oxidative Stress, Apoptosis, Ovary

P-76: Evaluation of DNA Methyl Transferases Enzymes in Sperm Preparation by Density Gradient Centrifugation

Rashidi M[1*], Tavalaee M[1], Nasr Esfahani MH[1, 2]

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: moloud.rashidi@gmail.com

Background: Sperm DNA methylation is a key point in keeping integrity of the genome and controlling gene expression. The level of global DNA methylation in sperm are higher than other male germ line in the testis and recent researches show that individuals with changed DNA methylation level in their sperm cells are infertile despite having normal sperm parameters. Methylation of DNA occurs by DNA methyl transferases enzymes (DNMTs) and until now, three kinds of DNMTs were recognized. DNMT1 is a maintenance DNMT, while DNMT3a and 3b are de novo DNMTs. Considering there is a close association between hypermethylated sperm with sperm quality, and DNMTs have important role in this phenomenon, therefore, the aim of this study was to compare DNMTs between fresh semen sample and processed samples with by density gradient centrifugation (DGC) method.
Materials and Methods: After collecting semen samples, each sample is divided into two parts. One part washed by PBS and other part processed by DGC. Percentage and intensity of DNMT1, DNMT3a and DNMT3b enzymes was assessed in each part by flow cytometry and results were compared between two groups.
Results: Percentage of sperm with normal morphology and motility significantly were higher in DGC method than fresh samples. Percentage of DNMT1 and DNMT3a were also significantly higher in DGC procedure than the fresh samples, while significant difference was not observed between two groups. In addition, significant difference was not observed in related to intensity of DNMTs.
Conclusion: Our result clearly showed that sperm preparation by DGC procedure in addition to separate normal sperm with high motility and morphology, it also can separate high percentage of sperm with DNMT1 and DNMT3a that have important roles in maintenance and de novo DNA methylation.
Keywords: Infertility, DNA Methylation, DNA Methyl Transferases, DGC, Sperm Preparation

P-77: *In Vitro* Development of Parthenogenetic Zona-Free Goat Embryo after Vitrification-Warming: A Possible Model

Rouhollahi Varnosfaderani Sh[*], Hosseini SM, Ostadhosseini S, Moradi R, Nasr Esfahani MH

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh_nasr@med.mui.ac.ir

Background: Zona free embryos are more sensitive to cryopreservation in all of species and in the all embryo developmental stages. In this study, we aim to introduce cryopreservation protocol for vitrification-warming of parthenogenetic zona-free goat embryo.
Materials and Methods: Goat collapsed expanded blastocysts were used for vitrification-warming with and without zona. Briefly, blastocyst was put in equilibrium medium for 6 min and then, before immersion in liquid nitrogen, embryos were held in vitrification medium for 35 sec. The embryos were warmed in the warming medium which contains 1 and 0.5 mol/l sucrose for 1 and 3 min, respectively. Finally, embryos were washed in the base medium and cultured for 48 hours in synthetic oviductal fluid. The percentage of re-expansion in two experimental groups was statistically evaluated.
Results: There was no significant difference between re-expansion percentage of two experimental groups and embryos quality.
Conclusion: Parthenogenetic Zona-free goat embryo and our cryopreservation protocol can be used as a model for optimizing vitrification-warming of zona-free embryos in assisted reproductive techniques.
Keywords: Cryopreservation, Zona Free Embryo, Asisted Reproductive Techniques

P-78: Olive Leaf Extract Promotes Sperm Quality in STZ-Induced Diabetic Rats

Salahi P[1*], Alirezaei M[2], Kheradmand A[3], Azizi A[1]

1. Students Research Committee, School of Veterinary Medicine, Lorestan University, Khorram Abad, Iran
2. Division of Biochemistry, School of Veterinary Medicine, Lorestan University, Khorram Abad, Iran
3. Department of Clinical Science, School of veterinary Medicine, Lorestan University, Khorram Abad, Iran
Email: pouyasalahi.veterinarian@gmail.com

Background: Diabetes mellitus represents one of the greatest threads to global health in the 21st century. In respect to, glucose metabolism is an important event in spermatogenesis, an important complication of diabetes is the disturbance in the male reproductive system. In this regard, many studies in both human and animals have confirmed the deleterious effect of diabetes on sexual functions. The aim of the present study was to evaluate the olive leaf extract effects on streptozotocine (STZ)-induced diabetes and to examine its modulatory effects on sperm quality.
Materials and Methods: Twenty adult male Sprague-Dawley rats were divided into four equal groups. The first group served as untreated control. Groups 2, 3, and 4 of rats were injected intraperitoneal STZ (65 mg/kg). The animals which exhibited blood glucose levels higher than 250 mg/dl by days 4-6, considered as diabetic rats. Groups 3 and 4 received olive leaf extract (100 and 150 mg/kg, orally) and vehicle to the control and diabetic rats for 10 consecutive days.
Results: Glycated haemoglobin percentage (%HbA1c) as a diabetic index significantly decreased in the animals ingested by the 150 mg/kg of the extract compared to the diabetic group ($P<0.001$). Olive leaf extract (150 mg/kg) could improve sperm quality of the treated rats against STZ deleterious effects in the diabetic rats ($P<0.001$), however, total sperm motility was significantly higher in the diabetic rats ($P<0.001$). Cholesterol concentration significantly increased in the diabetic and the extract-treated groups compared to the controls ($P<0.001$), and triglyceride level significantly decreased in the extract-treated animals (150 mg/kg) compared to the diabetic and the extract 100 mg/kg groups.
Conclusion: Our data suggest that olive leaf extract as a natural substance possesses beneficial antidiabetic effects on STZ-induced diabetes in rats and may be a good candidate to decline diabetes complication in men.
Keywords: Diabetes, Streptozotocine, Olive Extract, Rat, Testis

P-79: Effects of Cryopreservation on The Mitochondrial Membrane Potential of Rooster Sperm Using JC-1

Salehi M[1], Mahdavi AH[1*], Sharafi M[3], Shahverdi AH[2], Sharbatoghli M[2], Esmaeili V[2]

1. Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Poultry Science, College of Agriculture, Tarbiat Modares University, Tehran, Iran
Email: mahdavi@cc.iut.ac.ir; shahverdi@royaninstitute.org

Background: Evaluation of mitochondrial membrane potential with JC-1 has been performed for sperm cells in several species such as bull, human and equine; however, this method has not yet been reported for rooster sperm. The purpose of this study was to apply the JC-1 staining using flow cytometer procedure for evaluation of mitochondrial function of rooster sperm before and after cryopreservation in two freezing media (Lake vs. Beltsville).
Materials and Methods: Sperm samples were collected twice a week from three mature roosters. Then, sperm samples were pooled to eliminate the individual difference and subsequently diluted with a hand-made extender containing soybean lecithin. Samples were aspirated into straws, and subsequently cryopreserved in a one-step procedure. For assessment of mitochondria membrane potential 1.0 µL JC-1 was added to 500 µL of the sperm suspension containing 5×106. This experiment was replicated 3 times for fresh and frozen samples using two kinds of Extender Lake and Beltsville. Flow cytometry analysis of mitochondrial activity was performed by FACSCalibur with three replications.
Results: Although there was no significant difference for active mitochondria between lake (84.2 ± 1.89) and Beltsville (86.1 ± 1.89) in state of fresh, it was significantly when they compared to frozen state. Moreover, the percentages of rooster sperm with active functional mitochondria were reduced significantly after cryopreservation and this reduction was considerable in Lake Extender (32.9 ± 1.89) compared to Beltsville.
Conclusion: It seems that Beltsville extender based on soybean lecithin could be an efficient protectant for preservation of mitochondrial membrane potential in rooster sperm.
Keywords: Mitochondria, Rooster Sperm, Flow Cytometry, Cryopreservation

P-80: Assessment of Apoptotic Like Changes in Cryopreserved Rooster Sperm

Salehi M[1], Mahdavi AH[1*], Sharafi M[2], Shahverdi AH[3], Sharbatoghli M[3], Esmaeili V[3]

1. Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
2. Department of Poultry Science, College of Agriculture, Tarbiat Modares University, Tehran, Iran
3. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Emails: mahdavi@cc.iut.ac.ir; shahverdi@royaninstitute.org

Background: Phosphatidyl serine externalizations as indices of apoptotic like changes have been found to be enhanced during cryopreservation of sperm. Extenders may play an important role to reduce apoptotic changes in sperm due to stabilization effects on the sperm plasma membrane. Therefore, the purpose of this study was to assess the apoptotic like changes in rooster sperm after cryopreservation in two freezing media (Lake vs. Beltsville).
Materials and Methods: Semen samples were collected twice a week from three mature rooster. Then, samples were pooled to eliminate the individual difference and subsequently diluted with a hand-made extender containing soybean. Diluted semen were aspirated into straws, and subsequently equilibrated and then cryopreserved in a one-step procedure. For determination of externalization of phosphatidyl serine as an earliest indicator of apoptotic like changes in the semen sample, 10 ml Annexin

V–FITC was added to 100 ml of the sperm suspension and then, 10 ml of propidium iodide (PI; 1 mg/ ml) was added to be evaluated by flow cytometer. The sperm samples were classified into four groups: (1) viable nonapoptotic cells negative for both Annexin V and PI (A-/PI-); (2) early apoptotic cells positive for Annexin V but negative for PI (A+/PI-); (3) late apoptotic cells positive for both Annexin V and PI (A+/P+); and (4) necrotic cells negative for Annexin V but positive for PI (A-/P+). The late apoptotic and necrotic cells were categorized as dead cells. Flow cytometry analysis was performed by FACSCalibur flow cytometer in three replications.
Results: The significant percentage of live sperm (nonapoptotic) was observed in in the fresh of lake and Beltsville extenders compared to those in the frozen state. Moreover, the percentages of apoptotic and necrotic spermatozoa were significantly higher in the frozen extenders of lake and Beltsville respectively (27 ± 2, 30 ± 2), (29 ± 3.1, 30 ± 3.1) compared to those in the fresh of lake and Beltsville (11 ± 2.4, 10 ± 2.4), (9 ± 2.3, 14 ± 2.3).
Conclusion: Cryopreservation induces the apoptotic like changes in rooster sperm. Extender may reduce this failure due to protection characteristics.
Keywords: Apoptosis, Rooster Sperm, Annexin V, Flow Cytometry, Freezing

P-81: Animal Enriched Serum Contains Vitamin E and Fish Oil; A New Suggestion To Improve Frozen-Thawed Human Sperm Quality

Seifi S[1, 2*], Alizadeh AR[1], Shahverdi AH[1], Esmaeili V[1], Sharafi M[3], Sharbatoghli M[2], Maroufizadeh S[3]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Poultry Science, Faculty of Agriculture, University of Tarbiat Modares, Tehran, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: Cryo-injuries in human sperm cryopreservation encouraged researcher to design a suitable protocol for sperm freezing. Supplementation of freezing media with an enriched serum obtained from animal fed a diet supplemented with fish oil (FO) and vitamin E (VITE) could be a suitable strategy to preserve the quality of sperm after cryopreservation This study was performed to evaluate the effects of ram enriched serum contains vitamin E and omega-3 fatty acids on human sperm cryopreservation.
Materials and Methods: Sixteen rams divided into four groups and fed diets according to following dietary groups: Control (CTR), vitamin E (VITE; 200 IU/ram/day), fish oil (FO; 40 g/ram/day) and fish oil + vitamin E (FO+VITE). Afterward, sperm samples were collected from 20 healthy men at infertility clinic (Tehran, Iran). Samples were divided into six equal experimental groups for cryopreservation in freezing media (SpermFreezTM, Fertipro) containing 2.5% of animal enriched serum as follows: CTR, VITE, FO and VITE+FO. Moreover, 2.5% Fetal Bovine Serum (FBS) and control medium (SpermFreezTM with no additives) were used as control groups. several parameters such as motility with computer-assisted sperm analysis (CASA), viability (Eosin-nigrosin), DNA fragmentation with Sperm Chromatin Structure Assay (SCSA) and total ROS (Chemiluminescence assay) were recorded. Data were analyzed using SPSS and statistical differences among various groups were determined by ANOVA and Tuckey's post hoc test.
Results: The highest significant ($P<0.05$) percentage of sperm motility and viability were observed in groups containing VITE (40 ± 5.74 and 45 ± 8.03) and FO+VITE (35 ± 6.37 and 47 ± 7.74) compared to control group (29 ± 10.09 and 36.5 ± 10.47), respectively. Moreover, FBS produced the lowest significant ($P<0.05$) percentage of motility and viability (23 ± 6.90 and 31 ± 7.33) compared to control group, respectively. Interestingly, adding serum contains FO+VITE to freezing media improved curvilinear velocity (VCL) than other groups (53 vs. 31, 36, 37, 44, 48 μm/s for FO+VITE, FBS, FO, control, CTR, VITE, respectively). ROS concentrations were not significantly ($P>0.05$) affected by the serum supplementation. Flow cytometer parameters in this study confirmed our results related to motility and viability. Straight linear velocity (VSL) as well as Average path velocity (VAP) were changed by serum inclusion. This improvement is mostly related to characteristic of antioxidant activity of vitamin E and omega-3 fatty acids. This combination has several characteristic that may improve the quality of sperm such as membrane flexibility and improve signal transduction during cryopreservation.
Conclusion: It seems that low concentration of enriched serum contained FO+VITE can improved human sperm motility and viability after freezing-thawing. More investigations for roles of omega-3 fatty acids and their metabolites along with antioxidants need to be considered for protection of sperm against cryo-injuries in freezing media.
Keywords: Enriched Serum, Freezing, Sperm

P-82: Germ Cell Lineage Potential of Human Neonatal Foreskin stromal Cells

Soleimanpour- Lichaei S, Soleimanpour- Lichaei HR*

Department of Stem Cells and Regenerative Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: hrs@nigeb.ac.ir

Background: Male infertility has long been a difficult issue for many couples. However the successful differentiation of germ cell and live progeny from pluripotent stem cell bring the new hope to the couples suffering with infertility. The requirement of exogenous gene integration in process of reprogramming of iPS cells limits their application in reproductive medicine. Recently, several studies have demonstrated that human neonatal foreskin stromal cells (hNFSCs) possess multipotent characteristics, being able to differentiate to adipocytes and osteoblasts and potentially other cell types. Therefore, in a bid for infertility treatment, we examined the human neonatal foreskin fibroblast cells intrinsic ability to trans-differentiate into germ cell lineage.
Materials and Methods: Primary hNFSCs were isolated and cultured by explant organ culture method from newborn foreskin samples obtained at the time of circumcision from 7-14 day-old male donors. After establishing hNSSCs cell line, quantitative RT-PCR was then applied to assess the expression level of our target genes including those related to pre-meiotic and meiotic germ cell lineage.
Results: Our quantitative RT-PCR results indicated that hNFSCs possess an intrinsic potential to express pre-meiotic germ cell markers including Piwil2, C-kit, Stella, Itgα6, Hsp90, Oct4, and Itgβ1. However, the expression of meiotic and post-meiotic markers such as Scp3, Pgk2, and Prm1 were not detectable under

the standard growth condition which was applied in our study.
Conclusion: Our findings clearly demonstrated that hNFSCs possess an intrinsic potential to differentiate into pre-meiotic germ cell lineage including spermatogonial stem cells (SSCs) without genetic manipulation, providing novel approach for treatment of male infertility.
Keywords: Human Neonatal Foreskin Fibroblast, Male Infertility, Germ Cell Linage, Spermatogonial Stem Cell

P-83: Protective Effect of Matricaria Chemmomilla Extract on Histological Damage and Oxidative Stress Induced by Torsion/Detorsion in Adult Rat Ovarian

Soltani M[1], Moghimian M[1], Shokoohi M[2*], Abtahi SH[1], Shoorei H[2]

1. Department of Basic Sciences, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Email: a.shokoohy@yahoo.com

Background: This experimental study used a rat model to investigate the effect of an extract of Matricaria chemmomilla on histological damage and oxidative stress induced by torsion / detorsion in adult rat ovary
Materials and Methods: in our experimental study, were used 28 female Wistar rat. They randomly divided into 4 groups; G1, Sham group; G2, Ovarian torsion for 3 hours followed by detorsion 10 day(TDO); G3, Ovarian torsion for 3 hours and receiving 200 mg/kg hydroalcholic extracts of MC, 30min before detorsion and followed by detorsion (10 day) (TDOMC); G4, receiving 200 mg/kg hydroalcholic extracts of MC(MC) After the reperfusion period, 10 day, and their blood sampling, blood levels of estrogen, testosterone, some oxidative stress markers and anti-oxidant enzymes were assayed. Further assessment was carried out by histomorphometry at 10day post procedure.
Results: Comparison of The histological parameters showed a significant change in the G2 group as compared with other groups. The levels of estrogen, GPX, and superoxide dismutase significantly decreased in G2 group, and increased in G3 and G4 groups, and the malondialdehyde level increased in the duration of ischemia increased. Treatment by MC decreased the malondialdehyde level in G3and G4 groups.
Conclusion: The results of this study showed that Matricaria chemmomilla could be reduced oxidative stress and tissue damages following ovarian torsion/detorsion.
Keywords: Torsion/Detorsion, Tissue Damage, Oxidative Stress, Chemmomilla, Ovarian

P-84: Vitrification of Limited Number Sperm Using Microdroplet and Cryotop Techniques and Warming in Two Different Temperatures of 37 and 42°C

Tahmasebi M[1, 2*], Rezazadeh Valojerdi M[1, 2], Rashki Ghaleno L[2], Maroofizadeh S[3], Dalman A[2]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Anatomy, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Emails: mr_valojerdi@royaninstitute.org; mr_valojerdi@modares.ac.ir

Background: Cryopreservation of sperm plays an important role in preserving of male fertility, especially in men with a limited number of sperm. The aim of this study was the establishment limited sperm cryopreservation by petridish and cryotop with and without cryoprotectant agent; and checking the quality of sperm parameters, viability and DNA fragmentation after warming in two different temperatures.
Materials and Methods: 17 semen samples were collected from the normospermic cases. After swim up, progressive motile sperms vitrified on petridish and cryotop containing droplets of freezing medium, without commercial cryoprotectant solution containing HTF(Human tubal fluid) +sucrose+ taurine(S+T) or cryoprotectant solution containing HTF + sperm freeze (Cryoprotectant agent or CPA). After three days, each group was warmed in 37 and 42°C and at the first step was evaluated for parameters such as recovery, progressive motility and sperm viability to determine the best freezing method. At the second step, after selecting of best method for limited sperm cryopreservation, three other normospermic cases were processed, freezed and warmed in two different temperatures, and finally DNA fragmentation was assessed.
Results: In the microdroplet method, the total motility in (CPA)42 group was significantly higher than (CPA)37 and (S+T)42 groups ($P<0.05$). Also, the viability of sperms in (S+T)42 and (CPA)37 groups were significantly higher than (S+T)37 group ($P<0.05$). In cryotop method the total motility of (CPA)42 group showed a significant increase in comparison with (S+T)42 and (CPA)37 groups ($P<0.05$). As well, there was a significant difference between (CPA)37 and (CPA)42 groups total motility ($P<0.05$). The progressive motility and viability of (CPA)37 and (CPA)42 groups were higher than (S+T)37 and (S+T)42 groups, respectively. The evaluation of recovery and DNA fragmentation in cryotop method showed no significant difference between groups.
Conclusion: The limited sperm freezing by cryotop method is more efficient than Microdroplet and seems that the commercial cryoprotectant solution and warming in 42°C maintain the motility and viability of sperms in both methods.
Keywords: Human Sperm, Cryotop, Microdroplet, Warming Temperature, Cryoprotectant

P-85: Simultaneous Inhibition of TGFβ and Erk Pathways Promotes The Development of Mouse Single Blastomeres into Blastocyst

Yekani F[1, 2*], Azarnia M[1], Esfandiari F[2], Hassani SN[2], Baharvand H[2, 3]

1. Animal Biology, Biological Sciences, Kharazmi, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Developmental Biology, Science and Culture, Tehran, Iran
Email: baharvand@royaninstitute.org

Background: Blastomeres from 2- and 4-cell mouse embryos are proposed to be totipotent and have the potential for developing into blastocysts (BLs). However, the proper condition that prepares the requirements for single blastomeres (SBs) for further development is not identified. The aim of this study is improving the culture condition that support SBs for develop-

ing into BLs. Figuring out the culture condition that support SBs development would be helpful for patients who have one or low numbers of embryos to be transferred during assisted reproductive techniques (ART).
Materials and Methods: Blastomeres were isolated from 2- and 4-cell mouse embryos and cultured as SBs in drops of culture media. We examined, group culture vs. single culture of SBs and SBs were co-cultured with intact embryos either in T6 or T6+R2i media. The R2i compounds including SB431542 and PD0325901 inhibit TGF-β and MAP/ERK signaling pathways respectively. Results: Our results demonstrated that SBs can develop in conditions: single culture, group culture and co-culture with intact embryos with different efficiencies. Single culture of blastomeres supported blastocyst formation only in 1µl or lower volumes of media. 2-cell-derived SBs showed higher developmental rate compared to 4-cell SBs in single and group culture conditions. Furthermore, the more significant efficacy was observed in T6+R2i medium (P<0.001) in both culture condition. However, no significant difference was observed between 2 and 4-cell SBs developmental rate in co-culture with intact embryo. Based on Oct4 expression in inner cell mass (ICM) cells, ICM/trophoectoderm (TE) ratio was assessed for BLs; 4-cell SB-BLs have significantly higher ratio of ICM/TE compared to those of intact embryos (P<0.05).
Results: Our results demonstrated that SBs can develop in conditions: single culture, group culture and co-culture with intact embryos with different efficiencies. Single culture of blastomeres supported blastocyst formation only in 1µl or lower volumes of media. 2-cell-derived SBs showed higher developmental rate compared to 4-cell SBs in single and group culture conditions. Furthermore, the more significant efficacy was observed in T6+R2i medium (P<0.001) in both culture condition. However, no significant difference was observed between 2 and 4-cell SBs developmental rate in co-culture with intact embryo. Based on Oct4 expression in inner cell mass (ICM) cells, ICM/trophoectoderm (TE) ratio was assessed for BLs; 4-cell SB-BLs have significantly higher ratio of ICM/TE compared to those of intact embryos (P<0.05).
Conclusion: Our results demonstrated that 2-cell-derived SBs have higher developmental potential than 4-cell SBs. There is high association between BL formation and culture volume; lower volume of culture media was more effective for SB development compared with much volumes. One explanation is that high levels of autocrine/paracrine factors which are critical for development can be accumulated in lower volume of media. Moreover, our results showed that R2i-containing culture media promotes SB development. However, the implantation and pregnancy rate of SB-derived BLs should be determined and more studies are needed to prepare suitable conditions for human SBs for developing to BLs.
Keywords: Blastomere, R2i, Co-Culture, Autocrine/Paracrine, TE/ICM Ratio

P-86: Maternal Diet Supplementation by Omega-3 Fatty Acids and Vitamin E during Pregnancy and Lactation; Stereological Study on Offspring's Mature Testes

Zare Ebrahim Abad F[1*], Alizadeh AR[1], Hezavehei M[1], Esmaeili V[1], Kheymeh A[2], Najar M[3], Chehrazi M[4], Shahverdi AH[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Animal Core Facility, Reproductive Biomedicine Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: fahimehzare275@yahoo.com

Background: The objective was to determine how maternal nutrition effects on reproductive organs of mature male offspring.
Materials and Methods: Forty two mature female NMRI were divided in to 7 groups (n=6). The experimental groups consist of mothers who consumed several diets: I) Control (CTR; standard diet prenatal (PRE) and postnatal(POST) period); II) Prenatal–fish oil-vitamin E (PRE - FO+ VITE) gavages 0.01 ml/d/ mother FO + VITE 2×diet during prenatal period; III) (POST- FO+ VITE) gavages 0.01 ml/d/ mother FO + vitamin E 2× diet during postnatal period; IV) PRE-POST-FO+ VITE gavages 0.01 ml/d/ mother FO + VITE 2× diet during Pre and Postnatal Period; V) PRE-VITE; consumed VITE twofold greater than standard recommendations during prenatal period; VI) POST-VITE ;consumed VITE 2× during postnatal period; VII) PRE-POST–VITE; consumed VITE 2× during pre and postnatal period. After puberty, testis processed and stained with haematoxylin and eosin .The changes of testicular tissue were estimated using stereological methods and analyzed using SPSS.
Results: The mean total volume, volume of seminiferous tubules, length and diameter of seminiferous tubules in POST-VITE and POST- FO+VITE groups significantly increased compared than another groups (P<0.05). Also, the mean total volume in POST-VITE (106.38mm3) was higher than POST-FO+ VITE (98.28 mm^3) group (P<0.05). The height of the germinal epithelium seminiferous tubules increased in POST- FO+ VITE compared to PRE-POST–VITE and PRE- POST -FO+ VITE groups (94 vs. 83.70 and 64 Mm) (P<0.05).The thickness of the basement membrane in POST-VITE and POST-FO+VITE increased significantly compared with control group (6.32, 6.5 vs. 5.08 Mm) respectively (P<0.05).
Conclusion: Supplemented maternal diet with vitamin E or combination vitamin E with fish oil during lactation could improve testes tissue structure in mature offspring compared to supplementation maternal diet during pregnancy period.
Keywords: Maternal Nutrition, Fish Oil, Vitamin E, Testes

Ethics and Reproductive Health

P-87: Comparison Quality of Life in Infertile Men and Voluntary Childless Men

Ahmadi SE[1, 2*], Sajadi H[1], Rafiey H[1], Nosrati Nejad F[1]

1. IBRC, Academic Center for Education, Culture and Research, ACECR, Tehran, Iran
2. Social Welfare Management, Social Welfare and Health, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
Email: ahmadi@ibrc.ir

Background: Infertile men suffer from more psychological, emotional and sentimental problems than fertile men. On the other hand, there is a negative view toward fertile men who have decided against bearing a child in Iranian society which will affect their quality of life. This study was conducted to compare infertile men and childless fertile men in terms of their

health-related quality of life.
Materials and Methods: Forty-nine fertile men with no children and forty-two infertile men who met the inclusion criteria were entered into this descriptive study. All of the subjects were from Tehran. The accessible sample was selected by invitation. In all 91 participant were approached. Quality of life was assessed using the 36-item Short Form Health Survey (SF-36). The gathered data was analyzed using Statistical Package for the Social Sciences (SPSS) and Mann-Whitney U test and paired samples t test.
Results: Comparing the SF-36 scores between two groups represent that the quality of life in voluntary childless men is higher than infertile men. There was no significant relationship between the optionally childless group and compulsorily childless groups in terms of physical health, but the relationship between the former and latter in terms of mental health was significant.
Conclusion: A special attention should be directed toward different aspects of quality of life of infertile men and plans for improving their quality of life will improve their mental health.
Keywords: Quality of Life, Childlessness, Infertile Men, Tehran

P-88: Exploring Intervening Conditions for Start and Continuity of Assisted Reproductive Technology: A Qualitative Study

Ebrahimzadeh Zagami S[1*], Latifnejad Roudsari R[1], Janghorban R[2], Mousavi Bazaz SM[3], Amirian M[3]

1. Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Medicine, Medicine School, Mashhad University of Medical Sciences, Mashhad, Iran
Email: latifnejadr@mums.ac.ir

Background: Infertility treatment puts up infertile couples the cycle of hope and despair. Affecting factors in starting and continuation of infertility treatment.
Materials and Methods: This qualitative study was conducted at infertility center of Milad in Mashhad. Data were collected through semi-structured interviews with 10 infertile women and 4 infertile men and were analyzed with a qualitative content analysis technique.
Results: We found two categories that infertile couples encounter in start and continuity of infertility treatment: encouraging and limiting conditions. Hoping by others, observation of successful treatment, counterparts replication, self comparison with counterparts, interactions with others and family pressure were sub-themes for encouraging conditions. Financial problems, lack of physical access, woman's job, medical errors and interaction with couples with unsuccessful treatment were subthemes for limiting conditions.
Conclusion: This study showed that some conditions affect start and continuity of treatment in infertile couples. Identification of these factors could help policy makers to provide facilities for encouraging infertile couples to start treatment and maintain its continuity.
Keywords: Assisted Reproductive Technology, Encouraging Conditions, Limiting Conditions, Qualitative Research

P-89: Exploring Intervening Conditions Influencing The Issues of Commencement and Continuity of Using Assisted Reproductive Technologies: A Qualitative Study

Ebrahimzadeh Zagami S[1*], Latifnejad Roudsari R[2], Janghorban R[3], Mousavi Bazaz SM[4], Amirian M[5]

1. Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
2. Evidence-Based Care Research Center, Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Midwifery, School of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Social Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Department of Obstetrics and Gynecology, Milad Infertility Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: latifnejadr@mums.ac.ir

Background: Infertility treatment puts up infertile couples in the cycle of hope and despair. So acknowledgement of conditions affecting the process of infertility treatment seems salient. This study aimed to explore the intervening conditions influencing the commencement and continuity of using Assisted Reproductive Technologies in infertile women.
Materials and Methods: This exploratory qualitative study was conducted at Milad Infertility Center in Mashhad, Iran in 2016. Data were collected through semi-structured interviews with 10 infertile women and four infertile men and were analyzed with a qualitative content analysis technique.
Results: Two categories of encouraging and limiting conditions were emerged. Becoming hopeful by others' advice, observing others' successful treatment, comparison between self and others in successful ART attempts, positive interactions with others and family members' suggestions for following treatment were identified as encouraging conditions. Limiting conditions included financial problems, lack of access to physicians, women's employment, medical errors and interaction with couples with unsuccessful treatment.
Conclusion: This study showed that some encouraging and limiting conditions affect commencement and continuity of treatment in infertile couples. Identification of these factors could help policy makers and healthcare providers to provide facilities for encouraging infertile couples to start treatment and maintain its continuity.
Keywords: Assisted Reproductive Technology, Encouraging Conditions, Limiting Conditions, Qualitative Research

P-90: Fish-Oil Supplementation during Pregnancy

Farshbaf Khalili A[1], Jahanjoo F[1*], Ostadrahimi A[2], Heidarabadi S[3], Amini P[4]

1. Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Nutrition Research Centre, Tabriz Health Services Management Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Pediatrics, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: f.jahanjou@yahoo.com

Background: Growth of the fetus and small infants, especially

their brain during the last trimester of pregnancy and the first year of life is exceptionally fast and depends on the quality of their environment as well as the mother's nutrition. Fish oil contains long chain essential fatty acids (EFAs) of docosahexaenoic acid (DHA) (22:6 n-3) and eicosapentaenoic acid (EPA) (20:5 n-3). Long-chain polyunsaturated fatty acids (LCPUFAs) particularly DHA, are the most abundant fatty acids in the brain and essential for the growth and development of the brain and retina. This study aimed to evaluate the effect of fish oil supplementation on the development (primary outcome) and growth of 4-month-old infants.

Materials and Methods: This was a triple-blind randomized placebo-controlled trial carried out in Tabriz- Iran. The study population included 4-month- old infants whose mothers participated in the trial. One hundred and fifty women, being pregnant within the age of 18-35 years in first to fifth gravity, were randomly assigned into the following two groups: 1., FO supplementation containing 120 mg of DHA, 180 mg of EPA and 400 mg of ALA 2. or placebo with similar shape, size and weight containing liquid paraffin. Daily dose of FO or placebo capsules were 1000 mg once a day from the end of 20th week of pregnancy to 30 days after birth (about 24 weeks, 168 capsules). A self-report questionnaire was used to collect data about maternal socio-demographic and fertility characteristics. Neurodevelopment status of 4-month-old infants were evaluated by means of age and stages questionnaire (ASQ-2). Infants' weight, height and Head circumference measure was used for infants' growth.

Results: In the present study, consumption of FO supplements (1000 mg/d) from week 20 of pregnancy to 30 days after childbirth significantly improved the mean score of communication domain (adjusted mean difference 2.63; 95% confidence interval 0.36 to 4.89). Although the mean scores of neurodevelopment in all domains of the ASQ were higher in the supplemented group, but no statisyically significant differeces were observed between two groups in neurodevelopmental domains. Three (3.9%) subnormal neurodevelopment test (below 2 SD less than mean scores) were observed in the FO-supplemented versus 8 (11%) in the placebo group in different domains (adjusted odds ratio 0.33; 95% CI 0.08 to 1.32). There were no significant differences between the supplemented and placebo groups in terms of mean infant weight (adjusted difference, 0.11; 95% CI, -0.10 to 0.33), infant length (adjusted difference, ; 95% CI, -0.58 to 0.83) and infant head circumference (adjusted difference, -0.13; 95% CI, -0.47 to 0.21).

Conclusion: Fish oil supplementation is beneficial for the communication domain of neurodevelopment. Study results about the supplementation effect on other domains are inconclusive.

Keywords: Long-Chain Polyunsaturated Fatty Acids, Fish Oil, Pregnancy, ASQ-2, Infants

P-91: The Effect of Perinatal Fish-Oil Supplementation on Neurodevelopment and Growth of Infants: A Randomized Controlled Trial

Farshbaf Khalili A[1], Ostadrahimi A[2], Jahanjoo F[1*], Ghafari Z[3], Mohammadi M[4]

1. Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Nutrition Research Center, Tabriz Health Services Management Research Center, Tabriz University of Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. East Azerbaijan Provincial Health Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: f.jahanjou@yahoo.com

Background: Nutrition in pregnancy, during lactation, childhood, and later stages has a fundamental influence on overall development. Arachidonic acid (AA; 20:4n-6) and Docosahexaenoic acid (DHA; 22:5n-3) are essential for brain growth and cognitive development; they also accumulate rapidly in the brain and retina during the later stages of gestation and early postnatal life. In foods, the most important source of DHA is fish and fish oil. This study aimed to evaluate the effect of fish oil supplementation on the development and growth of 6-month-old infants.

Materials and Methods: In this triple-blind, randomized controlled trial, 150 pregnant women randomly were allocated into two groups, consuming daily fish oil supplementation (containing 120 mg docosahexaenoic acid and 180 mg eicosapentaenoic acid) or a placebo from week 20 of pregnancy to 30 days after childbirth. Infants' neurodevelopment was assessed using the Ages and Stages Questionnaire (ASQ). Infants' growth was measured using weight, length and head circumference.

Results: Although the mean scores of neurodevelopment were higher in the supplemented group than placebo group in each ASQ domain, a statistically significant difference was not observed. At the end of month 6, 4 (5.2%) subnormal neurodevelopment test (below 2 SD less than mean scores) were observed in the FO-supplemented versus 7 (9.8%) in the placebo group in different domains (adjusted odds ratio 0.33; 95% CI 0.08 to 1.32). However, these differences were not statistically significant. There were no significant differences in weight, length, or head circumference between the two groups of infants ($P \geq 0.05$).

Conclusion: Despite high scores in all neurodevelopment domains for supplemented group, lack of significance between the two groups, establish a need for further studies in this field with larger sample size, higher dose and with longer term follow up.

Keywords: Fish Oil, Neurodevelopment, ASQ-2, Infant

P-92: Mouse Maternal Omega-3 Dietary Fatty acid with or without Vitamin E Effects on The Offspring's Learning and Memory Function; A Practice by Y-maze Test

Ramezani M[1], Ghasemi ZS[1*], Alizadeh A[2]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
Email: sadatghasemi@yahoo.com

Background: The requirement of vitamin E is related to the dietary intake of fatty acids. Maternal omega-3 supplementation improves children's neurologic development and cognitive abilities. Docosahexaenoic acid (DHA) is essential for brain function and accumulates in brain membrane phospholipids. In behavioral science maze test used in animal cognition and working memory experiments. We evaluated the effect of dietary (n-3) fatty acid with and without vitamin E during gestation on offsprings' learning and memory function.

Materials and Methods: For this study 36 female mice divided in 6 groups and fed adlibitum during one week before

mating to weaning. The dietary groups were standard diet (control;C, 50 IU vit.E /kg diet), CLF and CHF groups which consumed 15 and 30 mg fish oil/100g of C diet, respectively. Moreover, a group received vitamin E supplemented diet (E, 125 IU vit.E /kg diet), ELF and EHF groups received 15 and 30 mg fish oil/100g E diet, respectively. All diets contain 3 percent oils which sunflower oil replaced with fish oil. After puberty, male and female offsprings were introduced to Y-maze test for 8 minutes. Number of total entrance and alternation percentage were analyzed with SAS.
Results: In female pups the number of total entrance decreased ($P<0.05$) in group E ($n=28 \pm 2.36$) and CLF ($n= 26 \pm 1.26$) than C ($n= 40 \pm 3.61$). Y-maze test analyses indicate that number of total entrance not affected by maternal diet in male pups. Alternation percentage did not differ in male and female pups.
Conclusion: Lower total entrance associate with effective memory function. Vitamin E supplementation during pregnancy and breastfeeding reduces number of total entrance in female pups and could alter learning and memory function. It seems this change affected by pup's sex which warrants further studies.
Keywords: Maternal Nutrition, Fish Oil, Vitamin E, Y-maze Test

P-93: Social Support and Quality of Life in Women Undergoing Assisted Reproductive Technology

Lotfi Kikoo S[1*], Omani Samani R[2], Ghaheri A[2]

1. Department of Psychology, Faculty of Humanities and Social Sciences, Islamic Azad University, Semnan, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: r.samani@gmail.com

Background: Infertility is considered a traumatic stressor for infertile couples, and failure to conceive becomes a psychosocial crisis for them. It has been reported that quality of life among infertile women is lower than fertile ones. Social support plays a key role in how an individual adjusts to a life crisis. The aim of our study was to evaluate the relation between social support in women undergoing assisted reproductive technology (ART) and their fertility related quality of life (FertiQol).
Materials and Methods: For this cross-sectional study, 350 infertile women were recruited by convenient sampling who were referred to the Royan institute for the first time and had no history of ART failure. This study was approved in ethical committee of Royan Institute. Social support was evaluated using the 12 item Multi-dimensional Scale of Perceived Social Support (MSPSS) questionnaire and quality of life was assessed by Fertility Quality of Life (FertiQol) with 34 items. P value<0.05 were considered as significant.
Results: Our results showed that infertile women with higher score of social support have significantly better quality of life ($r=0.41$ $P<0.001$). This relation was seen between all dimensions of social support with quality of life.
Conclusion: In conclusion, it can be advisable to consider social support as an important influencing factor on the quality of life among infertile women and higher this support by psychosocial counseling to the patients.
Keywords: Quality of Life, Social Support, Assisted Reproduction, Infertile Women, Infertility

P-94: The Perceived Stress Scale-10 Item: A Validation Study in Iranian Infertile Women

Maroufizadeh S[1*], Foroudifard F[2], Navid B[1], Mohammadi M[1], Ezabadi Z[1], Omani Samani R[1]

1. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Clinical Psychology, College of Humanities and Social Sciences, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: saman.maroufizadeh@gmail.com

Background: Infertility is a low control stressor with psychological consequences. The Perceived Stress Scale-10 Item (PSS-10) is one of the most frequently used instrument to measure perceived stress. The aim of this study was to examine the psychometric properties of the PSS-10 in Iranian infertile women.
Materials and Methods: This cross-sectional study included 240 infertile women in a referral fertility center in Tehran, Iran between February 2017 and March 2017. The PSS-10, the Hospital Anxiety and Depression Scale (HADS) were administrated to all participants. The psychometric properties of the PSS-10 were examined: construct validity using confirmatory factor analysis (CFA), reliability using Cronbach's alpha and convergent validity by examining the relationship with HADS.
Results: The CFA result indicated an acceptable fit of the data to the two-factor model of PSS-10 comprising Positive and Negative factors ($\chi^2/df=2.58$, GFI=0.93, CFI=0.96; RMSEA=0.081 and SRMR=0.061). Internal consistency of the scale was acceptable (Cronbach's alpha=0.842). The PSS-10 and its subscales were significantly correlated with anxiety and depression (all Ps<0.05), showing an acceptable convergent validity.
Conclusion: The Persian version of PSS-10 demonstrated satisfactory reliability and validity for assessing perceived stress in infertile women.
Keywords: Infertility, Perceived Stress Scale, Validity, Reliability, Persian

P-95: Evaluation of Coping Strategy in Infertile Couples Undergoing Assisted Reproduction Treatment

Mohammadi M[*], Omani Samani R, Sabeti Sh, Navid B, Amini P, Maroufizadeh S, Alizadeh A

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran , Iran
Email: maryammohammadi8766@gmail.com

Background: This study aimed to evaluate the coping strategy in infertile women referred to Royan institute, Tehran, Iran.
Material and Methods: In a cross-sectional study, the study sample consisted of 400 infertile couples, age at least 18 years and could read and write in Persian were enrolled at the Royan institute, Tehran, Iran, between 2014. Participants provided demographic and Ways of coping questionnaire (WOCQ). Data was analyzed by paired t test and multivariate analysis using SPSS software.
Results: Overall, 400 infertile couples participated. There was a significantly higher score for self-control in husbands compared to wives (P=0.016). As well as wives have lower score of Confronted Coping, Distancing than their husbands however Accepting Responsibility, Positive Reappraisal were lower in

wives than husbands but these differences are not significant (P>0.005). Mean score of Seeking Social Support and escape avoidance of wives was higher and significant. (P=0.037, 0.022 respectively).
Conclusion: Our finding showed that, husbands have more Problem focused coping style and wives have more Emotion focused coping style.
Keyword: Infertility, Coping Style, Couples

Female Infertility

P-96: Immunity response via MCP-1 in Tubal Ectopic pregnancy

Chekini Z[1*], Salehi Moghaddam Z[1], Madani A[1], Ramazanali F[1], Moini A[1, 3], Aflatoonian R[1]

1. Obstetrics and Gynecology Department , Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: R.Aflatoonian@gmail.com

Background: The innate immune system is the first line of defense against pathogens. Monocyte chemoattractant protein-1 (MCP-1) is one of the key chemokines that is a potent activator for monocytes/macrophages. MCP-1 plays a role in many chronic inflammatory and autoimmune diseases. Ectopic Pregnancy (EP) is a pregnancy where the fertilized ovum implants outside the uterus and more than 98% of them are located in the Fallopian tube (FT). The mechanisms by which these occur are to be primarily immunologically. This study aimed to evaluate the association between levels of MCP-1 expression in women with tubal pregnancy.
Materials and Methods: Ten women who underwent salpingectomy for EP have participated in this case-control study. Human chorionic gonadotropin (hCG) was injected in 14 days leading up to hysterectomy to produce a state of pseudo-pregnancy as a control group. Biopsies from infundibulum, ampulla and isthmus of FT were obtained from both groups. RNA isolation and cDNA synthesis from three region of FT were performed. RT-PCR was used to show the existence of MCP-1 expression in FT. Quantitative survey of MCP-1 expression was evaluated by using Q-PCR. Comparison between groups was done by analysis of mean ± SEM.
Results: The mean age of control and EP groups were 37.5 ± 5.36 years and 36 ± 5.69 years, respectively. Using RT-PCR shown that MCP-1 was expressed in three regions of FTs in case and control groups. Q-PCR has confirmed relative MCP-1 expression in all regions of FT carrying EP is lower than compared with normal fallopian tubes that receive hCG and these differences were statistically significant (P value ≤0.05).
Conclusion: MCP-1 protected against invading pathogens through recruitment of monocytes/macrophages. For the first time, we have shown that decrease in the expression of MCP-1 in fallopian tube might increase risk of EP and could be as predisposing factor in infections such as Chlamydia trachomatis.
Keywords: Ectopic Pregnancy, Fallopian Tube, Innate Immunity, Monocyte Chemoattractant Protein-1 (MCP-1)

P-97: Modeling *In Vitro* Fertilization Data in Multiple Points during The Cycle among Iranian Infertile Women

Ghaheri A[1*], Rasekhi A[1], Omani Samani R[2], Hajizadeh E[1]

1. Department of Biostatistics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: azadeh.ghaheri@modares.ac.ir

Background: Women undergoing IVF cycles should go successfully through multiple points during the procedure (i.e., implantation, clinical pregnancy, no spontaneous abortion and delivery) to achieve live births. On the other there is a need to consider previous reproductive outcomes and as well as the current cycle. In this study, data on multiple cycles and multiple points during the IVF cycle are observed for each individual to model the factors associated with success/failure in multiple points during IVF cycles in Iranian infertile women.
Materials and Methods: This historical cohort study includes 996 ART cycles of 511 infertile women. Generalized estimating equations (GEEs) was used for calculation of odds ratio (OR) and 95% confidence intervals (95% CI) of success in different points during the cycles. Clustered-weighted GEE (CWGEE) was also fitted to handle informative cluster size. Stata software, version 13 (Stata Corp, College Station, TX, USA) was used for all statistical analyses.
Results: After adjusting for potential confounders, receiving frozen embryo transfer was associated with higher odds of success compared to receiving fresh embryo transfer (adj OR, 2.26; 95% CI, 1.66-3.07); however, cycles with fresh embryo transfer did better in clinical pregnancy compared to those receiving frozen. Being in the age category of 38 to 40 was associated with lower odds of success compared to the reference category (<35) in CWGEE model (adj OR, 0.67; 95% CI, 0.45-1.00). Number of embryos transferred was positively associated with the odds of success in CWGEE (adj OR, 1.21; 95% CI, 1.03-1.42) as well as the GEE model.
Conclusion: Receiving frozen embryo transfer was positively associated with odds of success compared to cycles with fresh embryo transfer. Number of embryos transferred and women's age were significantly associated with odds of success.
Keywords: Generalized Estimating Equations, Cluster Weighted Generalized Estimating Equations, IVF

P-98: Effects of Prenatal Exposure to Electromagnetic Fields (EMF) on Estrogen and Progesterone Level in Female Rats

Gharamaleki H[*], Nikpour F

Department of Anatomical Sciences, Faculty of Medicine, University of Medical Sciences, Tabriz, Iran
Email: Hamideh.Gharamaleki@gmail.com

Background: During the 20th century, the exposure to electromagnetic fields (EMF) became an important source of concern about the possible effects in the living organisms. Electromagnetic fields interact with human tissues and may have adverse effects on reproduction. Some efforts have been made recently to investigate the incidence of ELF-EMF on human and animal physiology and behavior. The possible health effects of magnetic fields on reproduction and development have been ex-

tensively studied; however; the results of similar studies have often differed markedly from one another. This study evaluated the effects of electromagnetic fields in the prenatal period on estrogen and progesterone level in female rats (F1 generation).
Materials and Methods: In treatment group pregnant Wistar rats were exposed 3mT EMF for 21 days, 4 hours per day, in sham group pregnant rats in the similar condition but off the electromagnetic field and in the control group pregnant rats maintained in the room condition. After delivery 6 females neonate from each groups for studying changes estrogen and progesterone were kept up in standard condition to 12 weeks age, then eye blood samples of adult female and serum was kept in -80 oC freezer. Concentration of mentioned hormones that were estimated by the electrochemiluminescence- immunoassay (ECLIA) kits. The data were analyzed with SPSS v.19 by using T test and P<0.05 is considered as significant throughout this study.
Results: Biochemical analysis showed that there are not significant difference of prenatal effect of EMF on estrogen and progesterone in treatment group in comparison with sham and control group (P>0.05).
Conclusion: The EMF was, is and will be a part of our life. The progress of science will provide the world with new EMF emitting technologies and subsequently with new problems. The evaluation of the possible effects of EMF on the living organism is a complex process that needs the combined contributions of many scientific disciplines. The our results are in agreed with some studies that showed there is no significant change in progesterone and estrogen level after the initial exposure of EMF. Also in other study, adult Wistar female rats exposed continuously to a 50Hz SLF-EMF for 3 months proges¬terone levels, and estrogen levels in relation to the varying periods of the estrous cycle were not significantly altered. Nevertheless our results is disagreed with some studies that showed exposure to EMF in human didn't cause alteration in serum progesterone but at the long term exposure the progesterone level decrease. These differences are probably due to the differences in the radiation dose, radiation time, frequency, energy, animal species and the way in which waves are administered. Humans in modern society cannot avoid various kinds of EMF during household and occupational activities, but should be aware of the biological hazard of EMF.
Keywords: EMF, Prenatal, Estrogen, Progesterone

P-99: The Effect of Oral Contraceptives on Pathological Characteristics and Survival of Patients with Endometrial Cancer

Ghojazadeh M[1], Jahanjoo F[2*], Farshbaf Khalili A[2], Mohammadi M[3]

1. Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Research Center of Evidence Based Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: f.jahanjou@yahoo.com

Background: Cancer of the endometrium (lining of the uterus) is the most common gynecologic malignancy in developed countries and the second most common in developing countries (cervical cancer is more common). In northern Iran, Iran's cancer belt, endometrial cancer is the most common. Endometrial cancer most common etiology assumptions derived from epidemiology and clinical studies include: environmental factors, genetic factors and hormones. Estrogen-progestin oral contraceptive pills (OCPs) is one of the hormonal risk factors. Iran young population leads to increases in the use of OCPs, as the birth control pills. Therefore, this study aimed to investigate the effect of these OCPs on endometrial cancer.
Materials and Methods: In this retrospective Cohort study (2004-2015), hospital records of 472 women with endometrial cancer referred to Taleghani Hospital, Tabriz University of Medical Sciences were studied.
Results: Two hundred and twenty-two (47.0 %) out of 472 participants were using OCPs and other 250 participants do not (non-OCPs). The mean age of participants was 55.20 ± 13.14 years. According to histological grading, the frequency of grad 1, 2 and 3 was 27 (7.3 %), 212 (57.6 %) and 129 (35.1 %), respectively. 86 out of 129 (66.7 %) patients in grad 3 were from non-OCPs and 33.3 % of them were from OCPs (P<0.001). The tumor pathologic size in all cases of the OCPs group were<= 2 cm, while predominantly (59.9 %) it was 2 cm or larger in the non-OCPs group (P<0.001). lymph nodes were observed in 107 (22.7 %) subjects, of whom 38 (35.5 %) was in OCPs group and 69 (64.5 %) in the non-OCPs group (P=0.007). From all cases 129 (46.6 %) were Estrogen receptors (ER+), 188 (39.8%) (PR+), 164 (34.7 %) expressed both ER and PR (ER+, PR+) and 104 (22.0%) expressed no ER nor PR (ER-, PR-). ER-/PR- were predominantly in non-OCPs group (63.5 % vs. 36.5 %; P value = 0.044). In the present study, the mean survival time was 115.78 ± 2.24 months. The eleven years' survival rate in the OCPs group was significantly higher than non-OCPs group (100.0 % vs. 67.2%; P<0.001). In Cox proportional hazards model, tumor size of 2 cm or larger, lymph nodes involvement and not using OCPs were identified as the factors affecting survival time of these patients.
Conclusion: In addition to controlling the tumor size, OCPs use can improve survival time of patients with endometrial cancer.
Keywords: Endometrial Cancer, Survival Analysis, OCP

P-100: The Effect of Metformin and Glibenclamide on Endometrial Pathological Changes in Women with Polycystic Ovary Syndrome Undergoing Infertility Treatment: A Randomized Controlled Trial

Ghojazadeh M[1], Farshbaf Khalili A[2], Mohammadi M[3], Alizadeh A[3*]

1. Research Center of Evidence Based Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: St.Alizadeh@gmail.com

Background: Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder affecting 4–12% of women and also the most controversial. The Effects of metformin combined with merional and glibenclamide along with HCG for treating infertility due to polycystic ovary syndrome has been proved. But the pathological effects of these treatments remain controversial. This study aimed to compare the effects of metformin and glibenclamide on endometrial pathological changes

in women with polycystic ovary syndrome undergoing infertility treatment.
Materials and Methods: In this clinical trial, 50 infertile women with PCOS recruited in the study were randomly divided into two treatment groups (n=25 and n=25). The first group was treated with metformin at a dosage of 500 mg three times daily plus merinal based on which the second group was treated with glibenclamide at a dosage of 10 mg twice daily plus HCG for six months.
Results: Fifty patients with mean age of 38.04 ± 4.37 years, median parity of 2 ranging from 0 -7 and median abortion of 2 ranging from 0 - 4 were recruited in the study. Before the intervention, in dysfunctional uterine bleeding the most common histological pattern of endometrium includes disordered proliferative endometrium (DPE) 28 (56.0 %) followed by endometrial hyperplasia 22 (44.0 %) subjects. In the first group the frequency of DPE and endometrial hyperplasia was 16 (64.0%) and 9 (36.0 %), respectively and 12 (48.0 %) and 13 (52.0 %) in the second group (P=0.254). After the intervention, the histological pattern changed to atrophic endometrium in 36 (72.0 %), proliferative endometrium in 8 (16.0 %) and DPE in 2 (4.0 %) subjects. Ten out of 16 (62.5 %) DPE in the first group and 9 out 12 (75.0 %) in the second group changed to the atrophic endometrium. Five out of 9 (55.6 %) endometrial hyperplasia in the first group and 12 out 13 (92.3 %) in the second group changed to the atrophic endometrium, however, there was not a significant difference in histological pattern between two groups (P=0.302). Although Hematocrit increase was higher in the first group but it was not significant (1.35 ± 0.38 vs. 1.06 ± 0.25; P=0.535). Glibenclamide plus HCG resulted in greater thickening of the lining of the uterus as compared to metformin plus merinal (79.57 ± 3.22 vs. 86.07 ± 4.86; P=0.172).
Conclusion: Glibenclamide plus HCG resulted in better histological pattern changes but greater thickening of the lining of the uterus as compared to metformin plus merinal. Ambiguous results and lack of significance between the two groups establish a need for further studies in this field with larger sample size, higher dose and with longer term follow up.
Keywords: PCOS, Histological Pattern, Metformin, Glibenclamide, Merinal

P-101: Investigation of Cervix Mechanical Function in A Pregnant Woman to Understand Preterm Birth Development: A Functional Tissue Engineering Study

Jalalian Sedaghati Sh*, Vahidi B

Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
Email: bahman.vahidi@ut.ac.ir

Background: Preterm or premature labor is defined as deliveries before 37 weeks of pregnancy. Premature labor occurs in about 12% of all pregnancies. Complications of Preterm birth are responsible for approximately 35% of the world's 3 million perinatal deaths each year. preterm birth also affects growth and development of the brain and central nervous system functioning such as increased risk of cerebral palsy, developmental delays, impaired learning and visual disorders, and an increased risk of chronic disease in adulthood. Due to the two processes that are required to initiate labor, uterine contractions, and remodeling of the cervix, Parturition is recognized as a mechanical process. Premature cervical softening and subsequent cervical shortening is hypothesized to cause preterm birth. Currently, there is a lack of understanding in the structural and material factors that influence the mechanical function of the cervix throughout gestation. In this study, a finite element model of the pregnant uterus, cervix, and fetal membrane was built based on magnetic resonance imagining data in order to examine the mechanical function of the cervix under the physiologic loading conditions of pregnancy.
Materials and Methods: A finite element model was used to study pregnant uterine and cervix. A two-dimensional image of the uterus was used and scaled so that the uterocervix is 35cm long and 23cm in diameter. The uterus, the cervix, and the bony tissue are modeled using a continuum, 2D, plane strain elements; and the uterosacral and cardinal ligaments are modeled using truss elements that can only be compressed. Also, the resistance of the cervical canal to opening under hydrostatic pressure was modeled using a series of spring elements. The Expansion of the amniotic sac causes the uterine wall to expand, therefore a hydrostatic pressure was subjected on the uterine wall. The mechanical properties of the cervix were obtained from reported *in vivo* pressure and diameter data. The model was analyzed under two hydrostatic pressures, selected based on pressures observed commonly in labor (45mmHg), and pressure during preterm labor (90mmHg). The cervix Young's modulus is 40 Mpa and Young's modulus of the uterus is 566 KPa. The finite element software, ADINA was used for the simulation.
Results: In order to obtain greater clinical relevance, the absolute change in the width of the cervix was evaluated rather than strain. The results showed two times more dilation in the cervical canal under the higher pressure, 95 mm Hg as compared to the lower pressure, 45 mm Hg. Results obtained from this simulation has a good accordance with the experimental data. It was noted that a 0.25 cm opening at internal orifice couldn't be detected digitally by clinicians. This simulation further highlights the clinical advantage of using such models in understanding the factors associated with changes in the cervical canal and the associated risk for preterm labor.
Conclusion: The goal of this study is to use this basic model to inspire novel approaches to delineate normal and abnormal cervical function in pregnancy. Several important factors, such as the pressure, cervix geometry, and cervical stiffness under physiologic loading conditions were analyzed and discussed in this study. The mechanical model can be applied to other tubular visceral organs where concomitant measures of pressure and diameter can be obtained for a better understanding of diseases and their evolution or treatment.
Keywords: Tissue Engineering, Biomechanics, Finite Element, Cervical Insufficiency, Preterm Birth

P-102: Intravenous Injection of Bone Marrow Mesenchymal Stem Cells Suppress Oxidative Stress and Apoptosis in Mice with Induced Polycystic Ovary Syndrome

Kalhori Z[1], Soleimani Mehranjani M[1], Azadbakht M[2], Shariatzadeh MA[1]

1. Department of Biology, Faculty of Science, University of Arak, Arak, Iran
2. Department of Biology, Faculty of Science, University of Kermanshah, Kermanshah, Iran
Email: kalhoriz@ymail.com

Background: Polycystic ovary syndrome (PCOS) is a common cause of infertility due to anovulation. Bone marrow mesenchymal stem cells (BMMSCs) have anti-apoptotic and anti-oxidant

properties which may inhibit oxidative s tress and apoptotic death. Since apoptosis and oxidative stress are involved in the pathogenesis of PCOS, we decided to investigate the effect of BMSCs transplantation on oxidative Stress and Apoptosis in mice with induced PCOS.
Materials and Methods: PCOS was induced through daily injections of testosterone enanthate (1 mg/100g S.C. for 5 weeks). NMRI mice (3 weeks old) were divided into 3 groups (n=6): Control, PCOS, PCOS +BMMSCs. BMMSCs were injected into the mice through the tail vein (106MSCs/30g body weight) at 1 and 14 days after induction of PCOS and mice were killed at 2 weeks after last transplantation. The serum levels of Malondialdehyde (MDA) and the antioxidant capacity were measured relatively using thiobarbituric acid (TBA) and ferric reducing antioxidant power (FRAP) assay. The apoptosis rate in the follicles was evaluated by TUNEL staining. Data were analyzed using one way ANOVA and Tuckey's test and the means were considered significantly different at $P<0.05$.
Results: The Serum levels of MDA and the percentage of TUNEL-positive cells significantly increased in the PCOS group compared to the control. these parameters significantly reduced to the control level in the PCOS + BMMSCs group. Antioxidant capacity also reduced significantly in the PCOS group when compared to the control while it significantly increased in the PCOS+BMMSCs group to the control level.
Conclusion: BMSCs transplantation could prevent apoptosis and ameliorate the oxidative stress markers in mice with induced PCOS.
Keywords: Polycystic Ovary Syndrome, BMMSCs, Stress Oxidative, Apoptosis, Mouse

P-103: Relation between Altered Expression of Interleukin 13 (IL-13) with Tubal Ectopic Pregnancy

Madani A[1], Salehi Moghadam Z[1*], Chekini Z[1], Ramazanali F[1], Moini A[1, 2], Aflatoonian R[1]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Obstetrics and Gynecology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: R.Aflatoonian@gmail.com

Background: Ectopic pregnancy (EP) occurred when an embryo implanted and grows outside of the uterus cavity. Dysfunction of immune system is a one possible mechanism in etiology of EP. Macrophages play a central role in the balance of the immune response that regulated by cytokines such as interleukin 13 (IL-13). Interleukin (IL)-13 is a Th2 cytokine related to immunological disease. The aim of this study is investigation IL-13 expression in FTs carrying EP.
Materials and Methods: In this study, ten women underwent salpingectomy for EP group. Control group was women with healthy tube underwent hysterectomy who injected with human chorionic gonadotropin (hCG) in 14 days leading up to hysterectomy to produce state of pseudo-pregnancy. Biopsies from three regions of FT including infundibulum, ampulla and isthmus were collected from EP and control groups. IL-13 expression was survey with RT-PCR. In addition, Q-PCR was used to compare quantitative expression level of IL-13 between two groups. The statistical analysis was done by Chi-square test and mean ± SEM.
Results: We have founded, IL-13 was expressed in infundibulum, ampulla and isthmus of both groups by using RT-PCR. Wile, using Q-PCR shown that, in all regions of case group IL-13 level expression was significantly lower than control group (P value ≤ 0.05).
Conclusion: Due to IL-13 is a one important cytokines that role in immune response balance, growing evidence suggests an important role for IL-13 in mediating several interactions occurring between the immune and reproductive system. It is likely that turning down of IL-13 expression in FT can susceptibility to ectopic pregnancy.
Keywords: Ectopic Pregnancy, Fallopian Tube, Immunity Response, Expression, Interleukin 13 (IL-13)

P-104: Comparing The Effect of Metformin and N-Acetyl Cysteine on Pregnancy Rate and Metabolic Parameters in Polycystic Ovarian Syndrome Patients Undergoing IUI: A Double-Blind Randomized Controlled Trial

Madani T[1*], Hosseini R[2], Sedaghat M[1], Jahangiri N[1], Ahmadi J[1], Salmanyazdi R[2], Maroufizadeh S[3]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: tmadani@royaninstitute.org

Background: This study was designed to compare the effect of metformin and N-acetyl cysteine on pregnancy rate and metabolic parameters in polycystic ovarian syndrome (PCOS) patients undergoing IUI.
Materials and Methods: In this randomized controlled trial study, a total of 23 eligible patients with PCOS were included during 2015-2016. Patients were randomly assigned to one of two groups of Metformin (1.5 g/day orally) or N-acetyl cysteine (1.8 g/day orally). The PCOS patients were included the study only if they had age<40 years, body mass index (BMI)<30 and normal histrosalpingography. Exclusion criteria were as follows: infertility factor other than PCOS; presence of ovarian cysts; endocrinological disorders; hyperlipidemia; renal disease; liver disease; asthma; bronchospasm and other underlying disease; use of any OCP, metformin. Gonadotropin and lipid-lowering medications during the last three months; use of alcohol and cigarette. Clinical pregnancy rate and metabolic parameters were Primary Outcome Measure.
Results: Both groups were comparable in regards to mean age, BMI, and infertility duration (all Ps>0.05). There were no statistically significant differences between the groups with respect to number and size of follicles, hCG administration day, endometrial thickness, IUI day, fern test result, hyperstimulation syndrome, rate of cycle cancellation, clinical pregnancy rate and miscarriage rate (all Ps>0.05). At post-treatment process, there were no statistically significant differences between two groups with regard to mean metabolic parameters and hormonal parameters by controlling the pre-treatment levels (all Ps>0.05).
Conclusion: Women with PCOS undergoing IUI have the similar chance of achieving clinical pregnancy following treatment with N-acetyl cysteine or metformin. It seems that Metformin has no priority in improving metabolic parameters and insulin resistance factors compared to N-acetyl cysteine. However,

more studies are suggested, particularly with larger sample size.
Keywords: Polycystic Ovarian Syndrome, N-Acetyl Cysteine, Metformin, Metabolic Parameters, Pregnancy

P-105: Relation between Altered Expression of Interleukin 13 (IL-13) with Tubal Ectopic Pregnancy

Madani A[1], Salehi Moghadam Z[1*], Chekini Z[1], Ramazanali F[1], Moini A[1, 2], Aflatoonian R[1]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Obstetrics and Gynecology Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: R.Aflatoonian@gmail.com

Background: Ectopic pregnancy (EP) occurred when an embryo implanted and grows outside of the uterus cavity. Dysfunction of immune system is a one possible mechanism in etiology of EP. Macrophages play a central role in the balance of the immune response that regulated by cytokines such as interleukin 13 (IL-13). Interleukin (IL)-13 is a Th2 cytokine related to immunological disease. The aim of this study is investigation IL-13 expression in FTs carrying EP.
Materials and Methods: In this study, ten women underwent salpingectomy for EP group. Control group was women with healthy tube underwent hysterectomy who injected with human chorionic gonadotropin (hCG) in 14 days leading up to hysterectomy to produce state of pseudo-pregnancy. Biopsies from three regions of FT including infundibulum, ampulla and isthmus were collected from EP and control groups. IL-13 expression was survey with RT-PCR. In addition, Q-PCR was used to compare quantitative expression level of IL-13 between two groups. The statistical analysis was done by Chi-square test and mean ± SEM.
Results: We have founded, IL-13 was expressed in infundibulum, ampulla and isthmus of both groups by using RT-PCR. Wile, using Q-PCR shown that, in all regions of case group IL-13 level expression was significantly lower than control group (P value ≤0.05).
Conclusion: Due to IL-13 is a one important cytokines that role in immune response balance, growing evidence suggests an important role for IL-13 in mediating several interactions occurring between the immune and reproductive system. It is likely that turning down of IL-13 expression in FT can susceptibility to ectopic pregnancy.
Keywords: Ectopic Pregnancy, Fallopian Tube, Immunity Response, Expression, Interleukin 13 (IL-13)

P-106: The Effect of Vaginal Sildenafil on The Outcome of IVF/ICSI Cycles in Patients with Repeated IVF/ICSI Failures: A Pilot Study

Moini A[1*], Ahmadi F[2], Zafarani F[2], Jahangiri N[1], Sadeghi M[3], Salmanyazdi R[3], Malekzadeh F[1, 4], Chehrazi M[4]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: a_moini@royaninstitute.org

Background: Endometrial growth depends on uterine artery blood flow and the importance of endometrial development on *in vitro* fertilization (IVF) outcome has been previously reported. Poor uterine perfusion has been proposed as a cause of implantation failure in patients undergoing IVF/ICSI cycles. Sildenafil citrate, a 5-specific phosphodiesterase inhibitor, enhances arterial vasorelaxant effects of nitric oxide by preventing the degradation of cGMP. The aim of this study was to investigate the effects of vaginal sildenafil on the outcome of patients with at least two or three times of unsuccessful IVF / ICSI.
Materials and Methods: In this pilot randomized placebo controlled double blind study which was conducted from February 2015 to August 2016 in Royan Institute a total of 56 patients less than 38 years old and a history of at least two or three previous failed IVF/ICSI cycles were studied. The inclusion criteria were the blood FSH level<10 mIu/ml and normal ovarian reserve (antral follicle ≥ 6-8), a history of endometrial thickness<9 mm on the day of hCG administration in previous failed ART cycles, and normal endometrial morphology. All patients with a history of PCOS, myomectomy, Asherman's syndrome and uterine abnormality were excluded from study. The conventional GnRH protocol was used for ovarian stimulation. The patients were randomly divided into three groups: vaginal sildenafil (100 mg/daily), a combination of placebo (vaginal supp.) and vaginal Sildenafil (100 mg/daily), and placebo alone (vaginal sup). All patients underwent color Doppler ultrasound (day 14 previous cycle) to investigate any abnormality in uterus and adnexa, and endometrial parameters including thickness, echopattern, uterine artery resistance index and pulsatility index were recorded pre- and post-treatment. The primary outcome measure were implantation rate, chemical and clinical pregnancy rates. For data analysis, SPSS version 20 software was used. To demonstrate the value of the attribute measured, the mean ± SD was used. Chi square test and one way ANOVA were applied to compare categorical and continuous outcomes between the study groups, respectively. In all tests, the significance level was considered less than 0.05.
Results: In this study, three groups were matched in age, body mass index, duration of infertility, cause of infertility, the pattern of the endometrial, and uterine artery resistance indices before staring the cycle. There was no significant difference in three groups in endometrial thickness in hCG injection day. The chemical and clinical pregnancy in women who received sildenafil (alone or in combination with placebo) showed a two-fold increase in comparison to those who just used placebo. This increase was clinically meaningful, but according to sample size, it was statistically non-significant. The results of our study showed that the implantation is also higher in women who received placebo + sildenafil compared than those in two other groups. Abortion rate was not statistically significant among the groups.
Conclusion: It seems that vaginal sildenafil may, conceivably, improve chemical and clinical pregnancy rates in patients with a history of repeated IVF failure. Further randomized clinical trials using oral or vaginal sildenafil with higher sample size are recommended.
Keywords: Vaginal Sildenafil, Infertility, Repeated IVF/ICSI Failures

P-107: Psychological Disorders and Sexual Quality of Life among Fertile and Infertile Women with Polycystic Ovary Syndrome

Navid B*, Mohammadi M, Maroufizadeh S, Omani Samani R

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: reza.samani@gmail.com

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women and a frequent cause of infertility due to anovulation. Irregular menopauses, hirsutism, acnes, and obesity are seen in women affected with 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 but the impact of infertility in these women has not been studied. Therefore, we investigated the effects of infertility on sexual quality of life and psychological disorders among women with PCOS.
Materials and Methods: Participants consisted of150 infertile women referred to the Royan institute and 150 fertile women referring to women's health and family planning clinic of Arash hospital, both are referral centers in Tehran, the capital of Iran, between 2015 and 2016. The sampling method was convenient and all the cases were PCOS by Rotterdam criteria. Women responded to the (SCL90) and sexual quality of life female (SQOL-F) questionnaires. Data were analyzed with SPSS software using Independent sample t test.
Results: In subscales of SQOL-F, only sexual repression was significantly higher in fertiles (P=0.044). There was significantly higher scores in infertile PCOs female comparing fertile PCOs women in all nine subscales of SCL90 containing Hostility, Anxiety, Obsessive-compulsive, interspersed sensitivity somatization, psychoticism, paranoid ideation, depression and phobic anxiety (P<0.001).
Conclusion: Psychological symptoms such as anxiety, depression and etc higher in infertile women than fertile women with PCO.
Keywords: Psychological Disorders, Sexual Quality Of Life, Polycystic Ovary Syndrome, Infertile , Fertile

P-108: Compare Netttle Root Regimen with Tamoxifen+ Letrozol Grugs on Fertility Rate in Treatment of Polycystic Ovary Syndrom

Rasekh Jahromi A*, Kalavani L, Alborzi M, Zareibabaarabi Z

Department of Obstetrics and Gynecology, Jahrom University of Medical Sciences, Jahrom University of Medical Sciences, Jahrom, Iran
Email: Drrasekh@yahoo.com

Background: The aim of this study is to compare the effect of nettle roots regimen with Letrozole + tamoxifen drugs on fertility rate in treatment of infertile polycystic ovary syndrome in Jahrom city.
Materials and Methods: Two groups patients, 15 individuals letrozole + tamoxifen, 15 individuals Nettle root. Medical group (2 tablets letrozole 2.5 mg +2 tablets tamoxifen 10 mg pills daily), Herbal (4 tablets urthidine 150 mg daily)from day 3 to day 8 cycles were prescribed. TVS was performed on 8 menstrual cycles. Based on the number, size of follicles and endometrial diameter is decided. If follicle size between 18 -25 mm, and the number was less than 14, HCG was injected.
Results: Endometrial thickness in nettle root groups and tamoxifen+letrozole groups had significant difference (P<0.05) respectively. There is no significant difference (P<0.05) in the second and third visit in endometrial thickness and number of right and left ovarian follicles in nettle root and tamoxifen+ letrozole groups. But the size of the follicles in nettle root and tamoxifen + letrozole was significant difference. Also, There is no significant difference in frequency of ovulation and mean number of mature follicles between two groups. pregnancy rate was 13.3%(2 patients) in Nettle root treatment, that one patients (6.6%) aborted in the first trimester, another patients (6.6%) pregnancy with alive fetus. Pregnancy rates in women undergoing drug treatment with tamoxifen+ letrozole was 3 (20%) that 2 alive singleton and other twin alive fetuses. There is no abortion in this group.
Conclusion: Nettle root causes increasing follicular size than letrozole+ tamoxifen groups. Nettle root with letrozole + tamoxifen or with HMG or after laparoscopy operation can be used for ovulation induction, because of accessible and cost-effectiveness, pregnancy rates is approximated in both groups.
Keywords: Nettle Root, Letrozole, Tamoxifen, Pregnancy

P-109: Do Dietary Intakes Differ between Overweight Polycystic Ovary Syndrome Women and Controls?

Shishehgar F[1]*, Ramezani Tehrani F[2]

1. Department of Midwifery, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: shishehgarf@yahoo.com

Background: A possible relationship between dietary consumption and occurrence of PCOS has been theorized, however few studies examined the dietary pattern of women with PCOS. The aim of this study was to compare the dietary intakes between overweight PCOS women and controls.
Materials and Methods: This was a case control study to compare dietary intake of 84 overweight women with PCOS and 80 healthy age and BMI matched controls. We selected overweight, premenopausal PCOS women. Androgen Excess Society criteria was used for definition of PCOS. Healthy controls were recruited from women attending clinics for gynecologic examination. We used a validated food frequency questionnaire (FFQ) and N4 food analyzer. Similar nutritional characteristics were used to form 15 food categories. Food items with glycemic index>70 were defined as food with high glycemic index (HGI). International GI table was used to calculate GI value for each food items. Glycemic load (GL) was computed using GL= GI × carbohydrate (g)/100. Data was analyzed using T test or Mann-Whitney to compare the means of two groups.
Results: The results illustrated that energy intake (PCOS=2572 ± 606.8, non PCOS= 2456.9 ± 496.2 kcal/day, P=0.2), Percent of energy from carbohydrate (PCOS= 57.1 ± 6, non PCOS= 58.8 ± 5.6), protein (PCOS= 10.4 ± 2.4, non PCOS= 10.6 ± 2.3), fat (PCOS= 32 ± 6.5, non PCOS= 30 ± 5.7) were similar in cases and controls (P>0.05). Cases consumed less vegetables than controls (PCOS = 236 (175-301), non PCOS = 321(240.6-420.5) gram/day, P=0.026). Compared with controls, overweight women with

PCOS consumed more food items with HGI (PCOS=96.38 (40.4-158.1), non PCOS= 84.3 (40.1-142.1), gram /day, (P=0.048). Glycemic index intake (PCOS= 60.7 ± 6.8, non PCOS= 60.2 ± 5.6, P=0.6) and glycemic load intake (PCOS= 177.2 ± 53.7, non PCOS= 170.3 ± 40.2, P=0.3) in overweight women with or without PCOS were comparable. Intakes of other food categories in cases and controls were similar.
Conclusion: There were subtle differences in dietary intakes between overweight PCOS women and controls. Overweight women with PCOS compared with controls, consumed more food items with HGI and less vegetables.
Keywords: Polycystic Ovary Syndrome, Dietary Intake, Glycemic Index, Overweight

P-110: The Effect of Vitamin D Supplementation on The Outcomes of Assisted Reproductive Techniques in Infertile Women

Taebi M[1, 2*], Abedi S[2], Nasr Esfahani MH[3, 4]

1. Midwifery and Reproductive Health Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Midwifery and Reproductive Health, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
4. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: taebi_mb@yahoo.com

Background: One of the effective factors in fertility health is the level of serum vitamin D. Some studies have confirmed the effect of vitamin D on improvement of assisted reproductive treatments. This study was conducted to evaluate the effect of consumption of vitamin D supplementation on fertility indexes and achieving clinical pregnancy in infertile women.
Materials and Methods: The present study was a double-blind clinical trial that was conducted on infertile women who referred to Fertility and infertility Center of Isfahan who were randomly allocated into two groups of vitamin supplementation receivers (42 participants) and placebo (43 participants). Six weeks after the intervention, all of the steps of ovulation induction and *in vitro* fertilization were conducted for all participants. The fertility rate and quality of the embryos were evaluated and results were analyzed using SPSS16.
Results: No significant difference was observed between the intervention and the control group regarding the mean of achieved oocytes, the rate of fertilization, and the rate of good quality embryos (P>0.05). But the endometrial proliferative quality (P=0.01), the rate of biochemical pregnancy (P=0.013) showed a significant difference between the intervention and the control group.
Conclusion: Results of the present study showed that consuming vitamin D supplementation was directly associated with the rate of biochemical pregnancy.
Keywords: Infertility, Assisted Reproductive Techniques, Vitamin D

P-111: Comparison of Knowledge and Attitude toward Fertility Preservation in Cancer Patients between Gynecologists and Embryologists

Vesali S*, Omani Samani R, Karimi E, Khodabandehlou M

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: samiravesali@yahoo.com

Background: Cancer treatment may impair fertility in pediatric and adult populations is an emerging aspect of quality of life in these population. However, use of Fertility preservation (FP) among cancer patients is dependent on decision making of the patients and FP providers prior to cancer treatment. The objective of this survey was to explore the knowledge and attitude of Iranian gynecologists and embryologists about FP services provided to cancer patients.
Materials and Methods: Gynecologists and embryologists who completed this survey were a convenience sample of 277 specialists who attended large and important international congresses across Iran between May and September 2016. A 13-item self-administered questionnaire included questions on knowledge (5 questions) and attitudes (8 questions) with demographic characteristics. Question types included yes/no, multiple choice and 4-point Likert scale (greatly, usually, rarely, never) for attitude and 4-point Likert scale (none, little, intermediate and a lot) for Knowledge assessment.
Results: The mean age of participants was 37.07 (11.40) years. About 20% (n=54) and 80% (n=223) were male and female, respectively. Nearly 31.4% of participants (n=85) were gynecologists and 68.6% (n=186) embryologists. A total mean score of participants in knowledge of use of FP options among cancer patients was 2.97 (0.62). The total mean scores of gynecologists and embryologists in knowledge of use of FP options among cancer patients were 3.03 (0.65) and 2.95 (0.61), respectively (P=0.333). Embryologists obtained the mean score of 3.06 (0.94) and gynecologists 2.57 (0.97) in a knowledge question on use of gonadotropin releasing hormones (GnRH) treatment among cancer patients. The difference was statistically significant (P<0.0001). Most of gynecologists (98%) and embryologists (91.7%) declared more need information about FP options. A total mean score of participants in attitude of factors influencing on use of FP options in cancer patients was 3.41 (0.56). The total mean scores of gynecologists and embryologists in attitude of factors influencing on use of FP options in cancer patients were 3.41 (0.86) and 3.40 (0.43), respectively (P=0.941). Gynecologists were more positive attitude than embryologists toward having child/children as an important factor in enjoying FP by cancer patients [3.51 (0.59) vs. 3.01 (0.86); P=0.202)], while embryologists more agreed with time limitation as a barrier of use of FP among the patients than gynecologists [3.27 (0.81) vs. 3.16 (0.94); P=0.355)].
Conclusion: This sample of Iranian gynecologists and embryologists need more information on FP options. Insights into the areas of knowledge deficit provided by this study can be used to inform strategies for disseminating treatment-related infertility and FP information to gynecologists and embryologists, and improving awareness to prevent loss of fertility.
Keywords: Fertility Preservation, Cancer, Knowledge, Attitude, Gynecologists

P-112: Effect of Salvadora Persica Extract on Vaginal Infection with Candida Albicans in Mouse

Yary Z[1*], Azadbakht M[1], Mikaeili A[2], Modarresi M[3]

1. Department of Biology, Faculty of Science, University of Razi, Kermanshah, Iran

2. Department of Medical Mycology, Faculty of Medical Sciences, Kermanshah, Iran
3. Department of Pharmacognosy and Pharmaceutical Biotechnology, University of Medical Sciences, Kermanshah, Iran
Email: z.yary2012@yahoo.com

Background: Vaginal candidiasis is a common infection in women caused by a fungus called Candida albicans.The most important side effects of vaginal candidiasis increased risk of pelvic inflammatory disease, infertility, tubal damage, ectopic pregnancy, preterm delivery and low birth weight infants.Antibiotics areused commonly to treat this infection.Antibiotic treatments have several problems including reversible, increasing drug resistance and decreased sensitivity to the drug mushroom. In addition to anti-fungal properties of medicinal plants for treat, the plant has no known side effectsand drug resistance. This study aimed to evaluate the antifungal effect of aqueous extract of Salvadora persica extract on vaginal infection by Candida albicans in mouse.
Materials and Methods: Salvador persica aqueous extract at concentrations of5, 10, 15, 20, 25, 30, 50, 75, 100, 150, 200, 250 mg/ml were prepared. Disk diffusion method and minimum inhibitory concentration (MIC) was used for investigation ofthe antifungal effect of aqueous extract on both standard strains ptcc: 5027 and fungi cultured on Sabouraud dextrose Agarlaboratory (*In vitro*).In this experiment, powder clotrimazole with concentrations of 5.0, 1, 2 and 4 were prepared and were used as reference treatments. NMRI-mice were divided into two main groups depending on the type of fungus. Each group into five subgroups: control, untreated infected with fungus, infected with fungus under treatment with the plant extract Salvadora Persica, infected with fungus under distilled water and infected with the fungus and treated with clotrimazole were divided.For vaginal yeast in mice, a fungal concentration of 1×10^6 was transferred withsamplerto vagina.
Results: A minimum inhibitory concentration of the aqueous extract for fungus strain and Salvadora Persica standard wasat a concentration of 75 mg/ml and 150 mg/ml fungi ($P\leq0$ / 05), respectively.In addition, the minimum inhibitory concentrationof clotrimazole for fungus standard strain and fungi Candida albicans was at a concentration of 2 mg/ml and 4 ug/ml.
Conclusion: Our results demonstrated that Salvadora persica could be considered as treatment of vaginal candidiasis. Ithas good antifungal activity compared to clotrimazoleagainst Candida albicans and can be controlled vaginal candidiasis.
Keywords: Salvadora Persica, Candidaalbicans, Mice

P-113: Evaluation of Hydrocortisone Effect on Junctional Genes Expression of Human Fallopian Tube

Zhaeentan Sh[1*], Amjadi FS[1], Zandieh Z[1], Joghataei MT[1], Bakhtiyari M[1], Aflatoonian R[2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive, ACECR, Tehran, Iran
2. Department of Anatomical Sciences, Iran University of Medical Sciences, Tehran, Iran
Email: R.Aflatoonian@gmail.com

Background: Breakdown of the uterine epithelial cell junctions is a key event to implant a blastocyst. Ectopic pregnancy (EP) implantation junction breakdown occur outside of the uterine cavity with around 98% implanting in the fallopian tube. To overcome this problem, there is a need for a method that would increase the cells connection in appropriate sites especially in fallopian tube. Recently it has been shown that corticosteroids such as hydrocortisone (HC) increase the junctional molecules genes expression in blood-nerve barrier. In this study, we investigated whether HC increase the adherence and tight junction gene expressions in human fallopian tube cell line to prevent EP occur.
Materials and Methods: Human fallopian tube cell line (OE-E6/E7) was cultured with four concentration of hydrocortisone (0 nM, 50 nM, 100 nM and 200 nM) by three durations (24h, 48h and 72h). The genes expression of junctional molecules was investigated by QRT-PCR and compared to control. The candidate genes were claudin3, desmoglein-1 and E-cadherin.
Results: All genes expression was detected in all groups. The mean relative expression of claudin3, desmoglein-1 and E-cadherin was increased in the concentration 0 nM, 50 nM and 100 nM respectively but, the concentration of 200 nM was decreased. The optimum HC treatment duration was 48h treatment for all three target genes expression and there were significant differences between 48h and 72h of HC treatment.
Conclusion: The obtained results show that HC therapy by 100nM for 48 hours lead an increasing in junctional molecules genes expression. By the present study time and dose dependently HC treatment can be partially useful for reducing the risk of ectopic pregnancy. The cell density was downtrend despite increasing the dose and duration of HC exposure. So maybe the occurrence of EP in patients who were treated by IVF method will increase due to the high stress of IVF process which leads to high systemic glucocorticoids.
Keywords: Ectopic Pregnancy, Human Fallopian Tube, Hydrocortisone, Junctional Molecules

Genetics

P-114: Toll - Like Receptors (TLRs) Expression in Women with Repeated Implantation Failure

Aghajanpour S[1*], Amirchaghmaghi E[1, 2], Ashrafi M[1], Aflatoonian R[1]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: r.aflatoonian@gmail.com

Background: Repeated implantation failure (RIF) is an unknown major barrier of infertility in otherwise healthy women. The maternal immune system appears to be crucial for successful implantation and maintenance of pregnancy. It was revealed that TLRs, as the major compartments of innate immune system have relevance in ovulation, sperm capacitation and fertilization and implantation. Therefore, this study aimed to evaluate expression of toll-like receptors, in women with repeated implantation failure.
Materials and Methods: Twenty eligible patients with RIF and 10 fertile women as control were allocated to different groups: case group (N=20) and control group (N= 10). Genomic evaluation using PCR array which confirmed by Real-time PCR was performed for all pipelled endometrial sample in the luteal

phase for both groups.
Results: TLRs gene expression was detected in endometrial samples of case and control groups. The mean relative expression of TLRs gene was higher in control compared to case group.
Conclusion: Our data suggested that inflammation responses following activation of different TLRs are crucial factor for appropriate implantation of embryo. It seems, the lower expression of TLRs in endometrium of RIF cases resulted in immune dysregulation that may affect in collapse of embryo implantation in women with repeated implantation failure.
Keywords: Infertility, Innate Immunity, Toll-Like Receptor, Repeated Implantation Failure

P-115: Chromosomal Analysis of Couples with Bad Obstetric History

Demirhan O*, Tanrıverdi N, Süleymanova D

Department of Medical Biology and Genetics, Faculty of Medicine, University of Çukurova, Adana, Turkey
Email: osdemir@cu.edu.tr

Background: Chromosome Abnormalities (CAs) are one of the most important reason of reproductive diseases. The aim of this study was to exhibit the frequency and nature of CAs which is associated with the Bad Obstetric History (BOH) in the south of Turkey.
Materials and Methods: This study was carried out in a total of 895 individuals including 360 couples and 175 single women having BOH and with various incomes were investigated for CAs using blood culture and chromosomal banding technique
Results: A total of 895 individuals with BOH were analyzed, cytogenetically. The chromosomal abnormality was found in 4.4% of the sample studied. The 3.7% of these CAs was structural aberrations, and also numerical CAs was 0.7%. Although in one couple it was the wife and husband who had an abnormal karyotype. Specifically, inversions were the most common karyotypes (1.6%) among the all cases. For example, inversion chromosome 9 was seen among structural anomalies (1.2%). In 6 cases (0.7%), translocations were demonstrated. The others structural CAs (1.5%) were determined with i(9q), fra(Xq28), fra(20%), small(Y), Yqh+ and several CAs variations. Aproximately, 0.7% of individuals with BOH have the numerical CAs and aneuploidies.
Conclusion: It was found out that abnormal karyotypes were present in 4.4% of patients with BOH, and associated to female and bad obstetric history. Also, our findings confirm that the structural CAs, such as translocations and inversions were associated with a higher risk of BOH. Therefore, in couples with BOH, chromosomal evaluation can have a diagnostic value.
Keywords: Bad Obstetric History, Chromosomal Abmormalities, Karyotype

P-116: Next-Generation Sequencing Data Analysis of a Family-Based Study of Male Infertility

Jafarnia M[1, 2], Akbari A[1, 2*], Carrera P[3, 4], Ferrari M[3, 4, 5], Almadani N[6], Mohseni Meybodi A[6], Gourabi H[6], Anvar Z[7], Totonchi M[6]

1. Department of Biology, Faculty of Science, Fars Science and Research Branch, Islamic Azad University, Marvdasht, Iran
2. Department of Biology, Faculty of Science, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran
3. IRCCS San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Unit of Genomics for Human Disease Diagnosis, Milan, Italy
4. Laboratory of Clinical Molecular Biology, Ospedale San Raffaele, Milan, Italy
5. Vita-Salute San Raffaele University, chair of Clinical Pathology, Milan, Italy
6. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
7. Infertility Research Center, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Email: m.totonchi@royaninstitute.org

Background: Nearly 50 % of male infertility cases remain idiopathic calling for a new approach to identify the cause. As there are familial cases with no apparent problems revealed in physical examination of patients, it has long been suggested that there are genetic defects involved in the pathogenesis of male infertility that are not yet determined. We identified an extended family with seven affected males all of whom are brothers or cousins resulted from consanguineous marriages. All patients have been diagnosed with teratozoospermia due to the high percentage of sperm with abnormal morphology, particularly head of the sperm, in their semen samples. Moreover, this phenotype of infertility has the lowest success rate in pregnancy achieved by assisted reproductive technology (ART). Considerable size of consanguinity combined with the total number of affected and the fact that they share the phenotype make this family a very interesting case for a family-based study.
Materials and Methods: Whole-exome sequencing (WES) was performed on six people of the pedigree including four affected and two unaffected who are parents to affected. Sequencing run was performed on Illumina nextseq 500 platform. Resulting FASTQ files were checked for quality via FASTQC software. Next, Burrows-Wheeler Aligner (BWA) was used to map the files against the hg19 reference genome provided by the UCSC Genome Browser. Output file is in the SAM format which stands for Sequence Alignment/Map format. This file is converted to BAM format which is the binary form of SAM, validated and sorted using the Picard tools package. In the next step, Genome Analysis Toolkit (GATK) was used to recalibrate the base quality scores originally reported by the sequencing machine in order to make more accurate variant calls. Next, variant calling was done using HaplotypeCaller provided by GATK and also Freebayes caller. The output file from variant callers is a VCF file which lists all high quality variations from the reference genome that are present in the samples. However, the effect of the variations on protein and the frequency of the variations in different populations must be determined. For this purpose, ANNVOVAR was used which provides the frequency of variants from databases such as dbsnp, ExAC and 1000 genomes and annotates them according to data from variant effect prediction tools.
Results: Annotated VCF file generated from the analysis of all samples were filtered based on type of variation (deletion, insertion, SNP and MNP), location of the variation (Exon, intron, UTR and splice site) frequency in public databases and different populations, score from prediction tools (damaging, probably damaging and not damaging) and conservation score of the region in which the variation occurred. Based on literature database review, a list of genes previously linked with male infertility was prepared and the remaining variants were again

filtered based on these genes.
Conclusion: According to the pedigree, we anticipated an inheritance pattern of autosomal recessive for the disease. Thus, the segregation analysis was based on homozygous variants which resulted in a short list of variants in genes previously linked with male infertility. The presence of these variants in samples will be confirmed with conventional PCR followed by Sanger sequencing.
Keywords: Next-Generation Sequencing, Whole-Exome Sequencing, Family-Based Study, Male Infertility, Teratozoospermia

P-117: Evaluating The Genetic Alterations in Exon 11 of DPY19L2 Gene in Iranian Infertile Men with Globozoospermia

Alimohammadi F[1*], Sabbaghian M[2], Hashemi M[1], Totonchi M[3]

1. Department of Genetic, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: Globozoospermia is a specific type of teratozoospermia, which characterized by the presence of a large majority of round spermatozoa devoid of an acrosome in the ejaculate. DPY19L2 was selected as the most plausible candidate gene because of its predominant testis expression. The most common genetic defect is a homozygous 200-kb deletion removing the entire of DPY19L2 gene in patients with total globozoospermia. Our aim is to study exon 11 in globozoospermic patients.
Materials and Methods: In this case-control study 62 blood samples were retrieved from globozoospermic patients and 40 normozoospermic men as control group. After designing specific primers for the break points and exons 11 in all patients, PCR reactions were done for each DNA sample. Ultimately products were analysed by sequencing to determine genetic changes of the mentioned area.
Results: Our results showed that the frequency of homozygous deletion carriers was 22 of 29 in total globozoospermic patients and none of the 33 partial globozoospermic patients had the whole DPYI9L2 deletion. PCR product sequencing result illustrated previously reported a single nucleotide variation in the intronic region with rs4105524 in 21 patients and 31 control. There was a significant difference between globozoospermic patients and control group (P value<0.047).
Conclusion: Based on the results, 35% of globozoospermic patients had whole DPYI9L2 deletion. Although previous studies reported several variations in exon 11 of DPYI9L2, we only have found one variation within the intron 11. Our data revealed that this location of DPY19L2 has no effect on globozoospermia and there is no association between exon 11 of DPY19L2 gene variation and occurrence of described disorder.
Keywords: DPYI9L2, Infertility, Acrosome, Globozoospermia

P-118: Evaluation of Palm Pollen Effects on Sex Hormones, Performance Sperm Parameters and Gene Expression of CYP19 an Infertile Men: A Clinical Trial

Allamehzadeh Z[1*], Izadi M[2], Fallahi S[2], Amirzadeh Shams Sh[4], Koolivand M[3], Ahmadzadeh N[1]

1. Department of Biology, Islamic Azad University, Arsanjan Branch, Arsanjan, Iran
2. Fertility and Infertility Research Center, Hormozghan University of Medical Sciences. Bandar Abbas. Iran
3. Molecular Medicine Research Center, Hormozghan university of Medical Sciences. Bandar Abbas. Iran
4. Department of Microbiology, Islamic Azad University North Branch, Tehran, Iran
Email: maliheizadi@yahoo.com

Background: There is little scientific evidence supporting the therapeutic efficacy of phoenix dactylifera (date palm) pollen on human male infertility. The purpose of this study was to determine the effects of date palm pollen on human male infertility. Therefore, the present study was to investigate the effect of date palm pollen on the pattern of CYP19 gene expression, semen parameters and hormones levels of infertile men.
Materials and Methods: All of 40 patients with male infertility and 10 healthy individuals who were able to fertility in the last two years and their semen analysis was normal were selected for this study. Before and after treatment of patients with date palm pollen powder, 400 mg/kg every day for one month, blood and semen samples were taken. Before and at the end of therapy we measured the hormones levels and sperm numbers, motility, volume, morphology, and evaluated the CYP19 gene expression level by quantitative real-time PCR.
Results: Date palm pollen significantly increased testosterone levels, sperm count, volume and sperm normal morphology in patients after treatment (P<0.05). Our findings showed decreased expression of cyp19 gene after therapy period and its down regulation is significantly associated with increased sperm count, sperm motility, testosterone levels and improved sperm morphology (P<0.05). According to our study, it was found that palm pollen can be the effect on fertility power and have potential effects on sexual function in men.
Conclusion: This is the first report to study the effect of date palm pollen on the mRNA expression pattern of CYP19 gene of infertile men. The present findings revealed that additionally, down regulation of CYP19 positively are correlated with estradiol hormone levels. In the last decades, a number of inhibitors are designed to target aromatase enzyme.
Keywords: Phoenix Dactylifera, Semen, Real-Time PCR, CYP19 Gene, Infertility

P-119: Evaluating Genetic Variants in MSMB Promoter Region and Their Role in Intrauterine Insemination Outcome

Bagherian E[1, 2*], Mohseni Meybodi A[2], Sabbaghian M[3]

1. Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: anahitamohseni@gmail.com

Background: Unexplained male infertility is a term to describe men whom all their routine diagnostic tests results are normal, however they are unable to cause pregnancy in a fertile woman. IUI (Intrauterine insemination) is often the first-line treatment for unexplained infertility. Many factors such as proteomic and genomic profile of sperm can predict IUI success rate. MSMB (Beta-microseminoprotein) is an abundant protein in seminal plasma which is located on sperm head and neck and has an inhibitory effect on spontaneous acrosome reaction. The impact of some polymorphisms in MSMB promoter region on infertility in men with abnormal semen analysis is proved. The aim of the present study was to evaluate the impact of MSMB promoter mutations on IUI success rate in unexplained infertile men.
Materials and Methods: A case-control study was conducted among 100 unexplained infertile Iranian men who referred to Royan institute for IUI. 50 of men whose wives did not achieve pregnancy after IUI as case group and 50 of men whose wives get pregnant after IUI as control group were enrolled in our research. All of the routine diagnostic tests for these infertile men and their wives was normal and there was no abnormality in their semen analysis. To study the genetic variations, PCR for promoter region of MSMB gene was performed using specific primers that amplified from -599 to +209 of the gene and followed by direct sequencing. 100 samples have been sequenced. The statistical analysis was done using Chi Square ($P<0.05$).
Results: An SNP with rs12770171 was detected in heterozygote form in 29.8% of the sample with positive IUI result and in homozygote form in 2.1% of them. This SNP was detected in heterozygote form in 43.8% of the sample with negative IUI result and in homozygot form in 4.2% of them. Another SNP with rs10993994 was detected in heterozygote form in 53.2% of the sample with positive IUI result and in homozygote form in 19.1% of them. This SNP was detected in heterozygote form in 29.2% of the sample with negative IUI result and in homozygot form in 25% of them.
Conclusion: This is the first report on MSMB promoter region in the group of unexplained infertile men whose wife has undergone IUI. Our results suggests that SNPs rs12770171 and rs10993994 cannot be related to IUI success in unexplained infertile men.
Keywords: MSMB Gene, Male Infertility, Intrauterine Insemination, Unexplained Infertility

P-120: Computational Analysis of DNA Methylation Data Predicts Dynamic Role of PKC-catalyzed Phosphorylation in Women with Endometriosis

Barjaste N[1, 2*], Shahhoseini M[2], Afsharian P[2], Khochbin S[3], Masoudi Nejad A[1]

1. CNRS UMR 5309 INSERM U1209, Advanced Biosciences, Grenoble Alpes, Grenoble, France
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Laboratory of Systems Biology and Bioinformatics (LBB), Biochemistry and Biophysics, University of Tehran, Tehran, Iran
Email: n.barjaste@ut.ac.ir

Background: Endometrial DNA methylation has an essential role in the pathogenesis of endometriosis. Comparing methylation in control and ectopic endometrial is an appropriate study in endometriosis. In this study, Infinium HumanMethylation 450K BeadChip arrays were used to explore DNA methylation.
Materials and Methods: Differentially methylated regions (DMRs) between 9 case samples which have endometriosis and 6 control samples were identified by the minfi package. 20620 genomic regions were identified that a little number of these regions did not align to any genes and remained aligned to 10676 genes. KEGG pathway and GO analysis were applied to discover the role of methylated genes on disease-related biological pathways.
Results: The result shows that PKC-catalyzed phosphorylation of inhibitory phosphoprotein of myosin phosphatase is highly enriched in pathway analysis. PKC-catalyzed phosphorylation of inhibitory phosphoprotein of myosin phosphatase was only reported as an associated pathway in eutopic lesions of baboon model of endometriosis, but this pathway has not been reported in endometriosis patients yet.
Conclusion: Our preliminary data predict the potential role of this signaling pathway in the pathogenesis of endometriosis; the finding needs further studies to determine its specific mechanism related to endometriosis.
Keywords: Endometriosis, Epigenetics, Differentially Methylated Regions

P-121: Reprogenetics Advances: A New Hope and Insight for Recurrent Miscarriages Management for Young Couples

Binaafar S[1*], Mahdieh N[2]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: nmahdieh@gmail.com

Background: Miscarriage is an important reproductive medicine challenge typically with a history of recurrence. It is responsible for 1–5% of clinically recognized and up to 40 % of no identifiable pregnancies loss. Several studies have attempted to provide its etiology. However, almost 50 % of couples present with unknown cause.
Materials and Methods: It is well recognized that genetics is one of the most remarkable factor that impacts on the chance of a healthy pregnancy. Also, it seems these couples are likely to a greater risk of having an affected child. According to some reprogenetics studies, this phenomenon is related to either parental genetic abnormalities or embryonic other intrinsic factors.
Results: In most cases, they are fertile couples who are phenotypically normal and carry balanced translocations. The incidence of translocations is almost eight times more common among couples who experienced recurrent miscarriages than in the general population, 5.2 % in comparison with 0.7%. This depends on the type of translocation, the chromosomes involved and the length of the translocated segment. Different cytogenetics studies indicated that occurrence of Robertsonian and reciprocal translocations in the affected population is considerably higher than the total population, 1 % and 0.1 % respectively. The most important issue is its independent of paternal age nature whereas aneuploidy rates in germ cells, oocytes and sperms, are mainly depending on the age.
Conclusion: Currently, we have much hope with PGS to achieve live healthy birth. PGD is the only hope for carriers who experience repeated spontaneous abortions or recurrent implantation failure to have a normal offspring. At present, high-throughput genetic testing such as Mate-Pair Next Generation Sequencing

(MP-Seq), aCGH/ NGS are powerful tools that facilitate this aim achievement particularly for young couples.
Keywords: Reprogenetics, PGS, Recurrent Miscarriage, Young Couples

P-122: Ovarian Stimulation Leads to Down Regulation of K+ ion Channel in Endometrium

Davoodinik B[1*], Movaghar B[2], Favaedi R[1], Ramazanali F[3], Shahhoseini M[1]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Emails: m.shahhoseini@royaninstitute.org, b.movaghar@royaninstitute.org

Background: Although ovarian stimulation is a promising approach, recent clinical studies have demonstrated that stimulation using exogenous hormones might be detrimental to pregnancy. Further investigation on the endometrial actions of estrogen and progesterone hormones is currently providing significant insight into the implantation process in women, strongly suggesting that an abnormal response to progesterone underlies infertility in some patients. Recent clinical and animal studies have shown that ovarian stimulation disrupts endometrial function and embryo development and adversely affects pregnancy outcomes. Despite the effects of hormones on the endometrium we have to use them in infertility treatments. An indisputably critical action of steroid hormones on endometrial stroma is decidualization. Many ion channels were found to be involved in this process and have an indispensable role in the regulation of endometrial receptivity. Unusual expression of ion channels can lead to impaired endometrial receptivity and / or implantation failure. K+ concentration in uterine cavity and K+ activity in endometrium have major role in embryo implantation. KCNQ1 imprinting gene encodes a voltage-gated potassium channel in epithelium of many organs such as endometrium and is hypothesized that have functional role in implantation. KCNQ1 is located in the region of 11 chromosome that there is a non-coding mRNA called KCNQ1OT1. DNA methylation of maternal promoter of KCNQ1OT1 causes the expression of the paternal allele. This event suppresses the paternal alleles of cis imprinted genes and leads to expression of maternal KCNQ1 gene. The aim of this study was to compare epigenetic and expression of KCNQ1 in endometrium of oocyte donors before and after ovarian stimulation.
Materials and Methods: Endometrial samples obtained from nine volunteer 22-35 year old fertile oocyte donors through window of implantation by pipelle. Samples collected from 9 oocyte donors at one cycle before ovarian stimulation as control group and then after ovarian stimulation as experimental group. Expression of KCNQ1 in endometrial samples was evaluated quantitatively by real-time PCR. Also DNA methylation levels on promoter of KCNQ1OT1 were evaluated by ChIP real-time PCR. Data were analyzed by Independent-Samples T test followed by Tukey's test using SPSS version 22 software.
Results: The data showed a significant decrease in mRNA expression of KCNQ1 gene in endometrium of oocyte donors after vs. before ovarian stimulation ($P \leq 0.01$) and DNA methylation significantly increased in regulatory region of KCNQ1OT1 in endometrium of oocyte donors after vs. before ovarian stimulation ($P \leq 0.05$).
Conclusion: These data revealed association between gene expression of ion channels and ovarian stimulation and also suggested the functional role of hormonal ovarian stimulation in mechanism of ion channels that may decrease the embryo implantation.
Keywords: Oocyte Donation, Embryo Implantation, KCNQ1, Ion Channel, Window of Implantation

P-123: Evaluation of Relation between rs16826658 of WNT4 Gene and Endometriosis Stages in Iranian Population

Dehghani Mahmoudabadi B[*], Zare Mehrjardi E, Seifati M

Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran
Email: Seifati@gmail.com

Background: WNT family is a large group of secreted glycoproteins encoded by 19 distinct genes involved in the WNT signaling pathway. WNT4 is the first signaling molecule which affects the cascade of events that culminates in sex determination, through local secretion of growth factors. Several large gene mapping studies have demonstrated an association between endometriosis and markers located in or near to WNT4. The aim of our study was to evaluate the frequency of the polymorphism rs16826658 between early and advance endometriosis. Stage of endometriosis was determined according to the Revised American Fertility Society classification.
Materials and Methods: Peripheral blood was collected from 101 patients and 126 controls, and genomic DNA was extracted from lymphocytes according to salting out method. Detection of the polymorphism rs16826658 was performed using RFLP-PCR. The selection of the polymorphism was based on their previously association with endometriosis in other populations, respective P values. A total of 101 cases were selected to take part of this case–control study, being subdivided according to the Revised American Fertility Society classification, minimal/mild endometriosis (stage I and II) and moderate/severe endometriosis (stage III and IV).
Results: Significant differences in the genotype distribution were detected between the stage III/IV patients with endometriosis and the controls ($P=0.011$) but not between the stage I/II patients with endometriosis and the controls ($P=0.887$).
Conclusion: In our population rs16826658 polymorphism was not significant in endometriosis compared to healthy ones, that may be due to limited tested population. So, we proposed more extensive study, to associate this SNP for detecting endometriosis women.
Keywords: WNT4, Endometriosis, Women Infertility, Polymorphism

P-124: Circulatory TIMPs/MMPs Expression as Potential Biomarkers for Preeclamptic Pregnancies

Etesami E[1, 2], Seidabadi S[1, 2], Favaedi R[1], Shahhosseini M[1], Ghotbizade Vahdani F[3], Ramezanali F[4], Ghaheri A[5], Nikukar H[2], Zamanian MR[1*]

1. Department of Genetics, Reproductive Biomedicine Research

Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Stem Cell Biology, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Obstetrics and Gynecology, Vali-Asr Hospital, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Department of Epidemiology and Reproductive Health, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: zamanzss@gmail.com

Background: Preeclampsia (PE) is a systemic maternal syndrome characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation. It affects 5–10% of all pregnancies and the only effective treatment for severe cases will be delivery of the placenta. The proposed pathogenesis includes defective endometrial invasion by trophoblastic cells which leads to incomplete remodeling of spiral arteries and thereby inefficient perfusion of feto-placental unit. As the PE is the leading cause of perinatal morbidity and mortality, introduction of biomarkers for early detection and monitoring of at risk pregnancies seems to be necessary. Currently circulating fetal nucleic acids released from the placenta are possibly providing an important source for such novel biomarkers. The balance between TIMP/MMP genes is considered as an important factor in degradation and remodeling of extracellular matrix as well as remodeling of placental and uterine arteries. In addition, altered placental expression of TIMPs and MMPs has been reported in moderate to severe preeclamptic women. In the present study we aimed to introduce potential biomarkers for PE through comparison of the expression levels of TIMPs/MMPs in preeclamptic women (using cell free fetal RNA) to normal pregnancies.
Materials and Methods: Peripheral blood samples were obtained from 20 women with PE (28-32 weeks of gestational age) and 20 matched normal pregnancies. Cell free fetal RNA was extracted from the plasma and the expression of TIMPs/MMPs was measured through quantitative PCR.
Results: The results showed that expression levels of TIMP1, 2, 3 and 4 as well as MMP-2, 9 and 15 were significantly increased in preeclamptic pregnancies compared to controls while the MMP-14 was significantly decreased in preeclampsia.
Conclusion: Altered pattern of circulatory TIMPs/MMPs expression is associated with preeclampsia and may be used as potential biomarker for early detection and monitoring of high risk pregnancies once confirmative studies provide collective and supportive data.
Keywords: Preeclampsia, Free Fetal Nucleic Acid, TIMP, MMP

P-125: Differential Epigenetic Profile of Histone H1 Variants in Impaired

Favaedi R[1*], Ebrahimi Pour Basabi A[1], Sadighi Gilani MA[2, 3], Shahhoseini M[1]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Urology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: m.shahhoseini@royaninstitute.org

Background: Successful spermatogenesis requires tightly regulated epigenetic events to produce unique chromatin remodeling. Various histones, including testis-specific histones, exist during spermatogenesis and some of them have been reported to play a key role in chromatin remodeling. H1T and H1T2 are linker histone H1 variants, contributed in chromatin condensation as well as regulation of specific genes through spermatogenesis. Replacement of these histone H1 subtypes and hyperacetylation of histone H4 tail, facilitate the replacement of histones with sperm chromatin condensing proteins of transition proteins (TNPs) and protamins (PRMs). The aim of this study was to investigate the potential epigenetic role of testis-specific histone H1 variants (H1T and H1T2) in (in)fertility of men.
Materials and Methods: For this respect consent was obtained from azoospermic infertile men referred to Royan Institute according local ethical approval then, testes tissue samples were collected through ART procedure. Based on pathological features, tissue samples divided into following three groups: complete maturation arrest, Sertoli cell only syndrome, and hypospermatogenesis as positive control (at least 30 samples in each group). Relative expression of H1T, H1T2, TNPs and PRMs were evaluated by qRT-PCR. Also total levels of mentioned variants on chromatin as well as incorporation of them into regulatory regions of TNPs and PRMs was evaluated by chromatin ELISA and ChIP-real time PCR, respectively.
Results: mRNA expression of H1T, H1T2, TNPs and PRMs showed significant decrease in spermatogenic failure groups of complete maturation arrest and Sertoli cell only syndrome compared to hypospermatogenesis group ($P<0.05$). Results of chromatin ELISA and ChIP were in accordance to expression profile of mentioned genes in the way that H1T and H1T2 showed decreased total levels on chromatin in both groups with spermatogenesis impairment vs. positive control ($P<0.05$). Also, ChIP data revealed decreased incorporation of these histone variants into regulatory regions of chromatin condensing genes of TNPs and PRMs in complete maturation arrest and Sertoli cell only syndrome groups vs. to hypospermatogenesis group ($P<0.05$).
Conclusion: The findings of this study imply significant association between altered levels of testis specific histone variants with impairment of spermatogenesis and male infertility.
Keywords: Epigenetic, Histone H1 Variants, Impaired Spermatogenesis

P-126: Assessment of Genetic Variations in Exon 2 of KIF3B Gene in Infertile Men with Immotile Sperm Defects

Heydari R[1, 2], Yaghmaei P[1], Mohseni Meybodi A[3], Sabbaghian M[2]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: One of the main causes of male infertility is defect in structure and function of sperm cells. Patients with immotile sperm disorder have immotile tail sperm with disorganized axonem, and a significant decrease in sperm counts. Numerous proteins are involved in sperm formation. One of these proteins is Kinesin Family member 3B (KIF3B), which recently its essential role in sperm intra-flagellar transport and intra-manchette transport in male mice has been demonstrated. So its gene, which called Kinesin Family member 3B (KIF3B), is an appropriate candidate gene in human studies. Exon 2 of KIF3B gene, codes one of the main domains (Coild -coil Domain) of the protein and is the location for binding IFT20 protein. The purpose of this study was to assess the genetic variations of exon 2 of KIF3B gene in infertile men with immotile sperm defect and controls.
Materials and Methods: In this study, 50 infertile men with immotile sperm defect and 50 normozospermic men as controls were recruited. To study the genetic variations, DNA was extracted from peripheral blood, then PCR sequencing was done. Finch TV software was used to analyze the sequencing results.
Results: Sequencing analysis results identified one single-nucleotide polymorphisms (A>T) in exon 2 which was found in heterozygote form in 20 patients. No mutations or SNPs was identified in controls.
Conclusion: Although our study was conducted to evaluate the genetic variations in exon 2 of KIF3B gene and the results revealed just one variation in this exon, due to the high expression of KIF3B gene in testis, and considering the fact that KIF3B is evolutionarily conserved and not many studies have been conducted about the exact role of this gene in human male fertility, evaluation of other exons of this gene is strongly recommended.
Keywords: Immotile Sperm, Male Infertility, KIF3B Gene

P-127: Assessment of Variations in Splicing Sites of Exons 23 and 78 of DNAH1 Gene in Infertile Men with Multiple Morphological Abnormalities of The Sperm Flagella

Hosseini SH[1*], Sabbaghian M[1], Mohseni Meybodi A[2], Sadighi Gilani M[1]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: Multiple morphological abnormalities of the sperm flagella (MMAF) syndrome is a defect which causes male infertility due to asthenozoospermia. MMAF syndrome defined by presence of spermatozoa with mosaic of flagella abnormalities. Recent studies revealed that mutations in DNAH1, an axonemal inner dynein arm heavy chain gene, are responsible for MMAF syndrome, notably some of these mutations are in splicing sites of DNAH1 .Purpose of this study was to evaluate the genetic variations of intron-exon boundary sites of 2 exons (23 and 78) of DNAH1 in MMAF patients.
Materials and Methods: In this study, 33 infertile men with MMAF syndrome and 33 normozospermic men as controls were recruited. Initially, DNA was extracted from peripheral blood, and after PCR reaction and sequencing the results of sequenced segments were analyzed by Finch TV and they were compared with data obtained from the Ensembl database.
Results: Sequence analysis results did not identify any mutations or single-nucleotide polymorphisms (SNPs) in intron-exon boundary sites of exons 23 and 78 of DNAH1 gene in patient and control groups.
Conclusion: Although our data revealed no genetic variations but according to recant study which conducted by Ray et al. (2016), it is strongly recommended that this study continues in familial cases with MMAF syndrome.
Keywords: MMAF, Male Infertility, DNAH1

P-128: Developing A Fast and Effective Method for Purification of Tenecteplase from Recombinant CHO Cells

Javid H[1*], Abolghasemi Dehaghani S[1], Amiri Yekta A[1], Sanati MH[2], Nasr Esfahani MH[3], Gourabi H[1]

1. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Department of Molecular Biotechnology, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: h.javid@royaninstitute.org

Background: Tenecteplase (TNKase), a tissue Plasminogen activator is an invaluable therapeutic protein with 527 amino acid residues is widely used for medical purposes. TNKase has been developed from t-PA by Site Directed Mutagenesis in order to improve its properties. Currently in Iran, recombinant TNKase is produced by CHO-C111 cells in DMEM medium supplied with %2.5 FBS. Developing a fast, reliable and cheap TNKase purification method for further medical uses is critical and challenging. In current study by practicing verity chromatography methods, we achieved highly pure TNKase.
Materials and Methods: In the first multi-steps method the supernatant of CHO-C111 TNKase producing cells after concentration and dialysis against 20 mM Sodium phosphate buffer pH 6.5 applied on Sephadex G-10 column to remove phenol red, then HiPrep CM FF 16/10 used to remove albumin in FBS by a 0-1 M NaCl ascending gradient in the same buffer. In the next step, active fractions applied on L-lysine HyperD column and pure TNKase eluted by a 0-200 mM ε-aminocaproic acid and 0-500 mM L-arginine in the same buffer. In the second single step method, the supernatant of CHO-C111 TNKase producing cells after concentration and dialysis applied directly on L-lysine HyperD column. TNKase eluted as mentioned above. In all steps TNKase determined by SDS-PAGE, activity assay, Dot-blotting and western blotting. The final purified protein verified by MALDI-TOF TOF.
Results: In the first method, 5.36 mg TNKase with 162 U/mg specific activity, 28.47 purification fold and 31% yield recovered, while in the second method, 6.3 mg TNKase with 184 U/mg specific activity, 32.48 purification fold and 38.98% yield recovered. SDS-PAGE analysis and MALDI TOF TOF validate the purity of TNKase.
Conclusion: Aiming to improve TNKase production, quality and significant decrease in purification steps and costs, using Serum Free CHO cell lines e.g., CHO-DG44 is suggested.
Keywords: Tenecteplase, CHO, Tissue Plasminogen Activator, Purification

P-129: Noscapine Inhibitory Effect on Human Endometriotic Tissue in Three-Dimensional Culture Model of Endo-

metriosis: Apoptotic Genes Expression and Reduced Nitric Oxide Secretion

Khazaei M[1*], Khazaei M[1], Nasr Esfahani M[2]

1. Fertility and Infertility Research Center, Kermanshah University of Medical Science, Kermanshah, Iran
2. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mkhazaei1345@yahoo.com

Background: Human endometrium is a unique tissue which plays central role in reproduction. Noscapine is a water-soluble alkaloid derived from Papaver somniferum (Papaveraceae family) showed anti-cancer activity and it is candidate for endometriosis treatment. The aim of present study was to examine the effect of different doses of noscapine on human endometriotic tissue in a three-dimensional culture.
Materials and Methods: In this *in vitro* study, human endometrial tissues from endometriosis patients (n=8) were taken and were divided to 5 groups. Control groups were only treated with M199 medium containing 5% fetal bovine serum (FBS), while test groups were exposed to media containing 10, 50, 100 and 200 μM of noscapine, for 21 days. The growth score, angiogenesis, the nitric oxide (NO) and the expression of apoptotic genes were accessed. Statistical analyses were performed by one-way ANOVA.
Results: The mean of growth score of endometriotic explants exposed to 0 (control), 10, 50, 100 and 200 μM of noscapine, were 2.2 ± 0.55, 1.7 ± 0.45, 1.44 ± 0.27, 0.29 ± 0.1 and 0.1 ± 0.08. Also, the mean of NO levels were 58.88 ± 15.06, 43.02 ± 14.86, 36.09 ± 12.27, 19.43 ± 4.99 and 14.20 ± 3.34, respectively with significant difference ($P<0.003$). In endometriotic tissues, the expression of p53, Bax, caspase 3 and caspase 8 were increased 1.5, 1.6, 2.7 and 1.3 folds, respectively, compared to control group. In addition, the expression of Bcl2 and Sirt1 was decreased
Conclusion: Noscapine showed a time- and dose-dependent inhibitory effect on human endometriotic tissue. The expression of apoptotic genes significantly increased as a dose-dependent manner while the levels of Bcl2 and Sirt1 reduced.
Keywords: Endometriosis, Noscapine, Apoptosis, Nitric Oxide, Sirtuin

P-130: Cloning and Expression of Recombinant Human Follicle Stimulating Hormone in CHO-DG44 Cells Using Human Serum Albumin Signal Peptide

Kimiai M[1*], Javid H[1], Daneshipour A[1], Norouzi F[1], Amiri Yekta A[1], Sanati MH[2], Gourabi H[1]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: mhsanati@yahoo.com

Background: Follicle Stimulating Hormone (FSH), a 35.5 kDa heterodimer glycoprotein, is secreted from anterior pituitary gland and affects reproductivity and pubertal development. FSH utilized as an infertility treatment, thus developing novel recombinant FSH (rFSH) production methods in CHO-DG44 serum free cells is highly demanded. Owning to efficient proliferation, post-translational modifications and later simplicity of purification strategies, CHO-DG44 is invaluable.
Materials and Methods: The pOptiVEC expression vector containing CMV promoter used for cloning of optimized FSH subunits genes that were ligated and jointed to DHFR gene as a marker via IRES sequence. Human serum albumin signal peptide was added to the construct to enhance the recombinant products secretion. The SignalP 4.1 Server used to predict the presence and location of signal peptide cleavage sites in amino acid sequences, and the prediction of the transmembrane helices checked by TMHMM Server v.2.0. Correct cloning approved by PCR and sequencing, then the linearized vector was transferred into dhfr- CHO-DG44 cells via FreeStyleTM MAX Reagent. The selection of transfected cells was done in CD OptiCHO™ medium and different methotrexate concentrations. Validation of gene expression was done by extracting the mRNA followed by cDNA synthesis and Real-Time PCR. The cells with high protein expression were selected, the protein profile checked by SDS-PAGE, Western Blotting and ELISA.
Results: The recombinant plasmid construct sequencing results showed the correct insertion and orientation of inserted elements. Moreover, the bioinformatics analysis of protein sequences confirmed the correct cleavage site of the signal peptide and it to be secreted. The colony PCR of transfected cells and RT-PCR confirmed the integration of construct into the host genome and mRNA expression level, respectively. The preliminary results of SDS-PAGE, Western Blotting and ELISA determined the expression and secretion of rFSH.
Conclusion: Using CHO-DG44 and human serum albumin signal peptide can enhance the therapeutic rFSH production and secretion, as a reliable method.
Keywords: FSH, Albumin Signal Peptide, CHO-DG44

P-131: Study of The Relation between Chlamydia Trachomatis Infection and Beta-Defensin 126 (DEFB126) Gene Deletion in Infertile Men Refered to Royan Institute

Maghareh Abed E[1*], Mohseni Meybodi A[2], Eghdami A[2], Sabbaghian M[3], Borjian P[3]

1. Deaprtment of Biology, Faculty of Basic Sciences, Islamic Azad University of Saveh, Saveh, Iran
2. Department of Genetic, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: elhammagharehabed@yahoo.com

Background: Chlamydia trachomatis(CT), an obligate intracellular bacteria, requires living cells to replicate itself. Half of men infected with CT are asymptomatic. CT infection can remain up to four years in the couple and affect their fertility. The relationship between CT and infertility is very important because most patients are asymptomatic and untreated. Most common symptoms of infection in men include urethritis, epididymitis, urinary tract inflammations and reduced sperm quality. One of the most important components of the Sperm Glycocalyx surface coating in human is DEFB126 protein. This polypeptide covers surface of sperm cells during pass through epididymis and has important role in Immune system. Human β-defensin 126 (12kDa) is a small cationic glycoprotein that is

highly rich of cysteine. DEFB126 gene is located on the subtelomeric end of 20p13 in human. It is considered as an important component of the human sperm glycocalyx and provides protection for sperms from infection-causing microbes and against the female immune system.
Materials and Methods: According to the role of DEFB126 against infection, the aim of this study was to investigate the frequency of deletion of two nucleotides in gene DEFB126 and its relation with the prevalence of CT in semen samples of Iranian infertile patients, referred to Royan Inst. In this study, among1080 patients with poor sperm parameters, were selected for primary screening and detecting, 155(14.3%) patients were diagnosed with ELISA test. The Sperm's DNA was extracted in order to confirm the presence of Chlamydia. Chlamydia genome amplification was performed using specific primers. Among these samples with CT, 50 patients of whom symptomatic and 50 patients were asymptomatic, were considered on cytosine dinucleotide deletion with Standard PCR، Sequencing and 70 fertile men with normal sperm quality and without any past history of CT infection were selected as controls.
Results: The results showed that among three type of genotypes seen in these cases, wt/wt, heterozygous wt/del and homozygous mutation del/del, males who had CT infection showed significantly higher had frequency of homozygous mutations del/del in DEFB126.
Conclusion: The results of this study demonstrated that, because of gene mutation in DEFB126 in Iranian infertile men with CT infection, the immunogenicity role of DEFB126 can be impaired. Accordingly, patients with this mutation are more susceptible and prone to develop infections such as Chlamydia Trachomatis.
Keywords: Male Infertility , Chlamydia Trachomatis , β-defensin 126 , Glycocalyx, Gene Mutation

P-132: Increase of Mean Relative Hypothalamic Ghrelin Gene Expression following Intra Cerebral Dopamine Hydrocloride Injection

Mahmoudi F[1*], Khazali H[2]

1. Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran
2. Department of Biology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
Email: faribamahmoudi_14@yahoo.com

Background: Ghrelin is one of the most important synthetized peptides in the central nervous system and peripheral organs under negative energy balance. It exerts inhibitory effects on sexual hormone secretions. Dopamine is a neurotransmitter which is secreted from hypothalamus and other areas of brain. it inhibits reproductive axis. In the present study the effects of dopamine were investigated on hypothalamic ghrelin gene expression.
Materials and Methods: Fifteen adult Wistar male rats in three groups(n=5 in each group) received saline, 5 or 15 microgram dopamine hydrocloride via third cerebral ventricle respectively. Mean relative ghrelin gene expression was determined by RT-PCR.
Results: Injection of 5 microgram dopamine did not significantly increased mean relative ghrelin mRNA levels compared to saline. While it significantly increased following 15 microgram dopamine compared to saline.
Conclusion: Ghrelin signaling pathway may be one of pathways involved in the exerting inhibitory effects of hypothalamic dopaminergic neurons on the reproductive hormone secretions.
Keywords: Ghrelin Gene, Dopamine, Rats

P-133: Lapatinib Treatment Decreases Preimplantation Aneuploidy Rate in IVF Embryos

Maleki P[1, 2*], Gourabi H[1], Bazrgar M[1]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Genetics, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: gourabi@royaninstitute.org

Background: One of the major reasons for implantaion failure and spontaneous abortion of embryos obtained from *in vitro* fertilization (IVF) is high incidence of chromosomal abnormalities in preimplantation development that mostly are diploid-aneuploid mosaic. We expect more sensitivity of preimplantation aneuploid embryos to lapatinib based on reports for more sensitivity of aneuploid cell lines to anticancer drugs. Lapatinib is a potent ATP-competitive inhibitor, which simultaneously inhibits both EGFR and HER2, and leads to marked inhibition of cell division with subsequent apoptosis. To examine this hypothesis which some anticancer drugs can inhibit mosaic embryo formation in *in vitro* culture, lapatinib was used for treatment of IVF embryos.
Materials and Methods: Nontoxic dose of lapatinib was determined by treatment of 132 late two-cell mouse embryos obtained through IVF with 0.02, 0.05, 0.1, 0.2, 0.5 μM of lapatinib for 12 and 24 hours. To evaluate the effect of drug on preimplantation development, 706 late two-cell embryos were treated with nontoxic dose of lapatinib upto day 2.5 post IVF. Development were followed upto day 4 for 529 embryos in control and treatment groups. On the fourth day, embryos' blastomeres were evaluated for aneuploidy using Fluorescent In Situ Hybridization (FISH).
Results: In terms of embryo development, 0.2 μM for 24h was determined as nontoxic appropriate treatment condition for further evaluations. Development to 8-cell stage in treatment group was lower than controls (16.99% versus 22.77%. P=0.054). There was no differences in reaching to blastocyst between treated and controls after day 4 (30.94% vs. 32.27%, P=0.741). FISH analyses revealed that the rate of aneuploidy in chromosomes 2 and 11 was 9.7% and 9.2% respectively in treatment group and 25.6% and 19.3% in controls (P=0.000). Frequency of normal cells was significantly higher in lapatinib group (85.9%) than control ones (67.3%, P=0.000). Sixty one percent of embryos in treatment group had more than 90% diploid blastomeres, that was much more than controls (14.6%) (P=0.000). Mosaicism rate was lower in treated embryos (61% vs. 85.4%, P=0.008).
Conclusion: Lapatinib significantly reduced aneuploidy without decrease in the rate of development to blastocyst stage.
Keywords: IVF, Preimplantation, Lapatinib, Blastomere, Blastocyst

P-134: Genetic Variation Analysis of HIST1H1T Regulatory Region in Non-Obstructive Azoospermic Men

Mollaee Zh[1, 2*], Mohseni Meybodi A[2], Shahhoseini M[2], Afsharian P[2], Zamanian MR[2], Sabaghian M[3]

1. Department of Molecular Genetic, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: anahitamohseni@gmail.com

Background: The proper conduct of spermatogenesis process is essential for male fertility. Histones are a family of essential proteins involved in DNA packaging. During normal process of spermatogenesis in sperm chromatin, histones are replaced by testis-specific histone variants. The gene encoding H1t (HIST1H1T), a testicular variant of histone H1, is expressed during spermatogenesis especially in primary spermatocytes, and facilitates histone to protamine exchanges during maturation of sperm. Regulatory region of HIST1H1T gene is the location for binding of some important transcription factors. The aim of the present study was to evaluate the genetic variations of HIST1H1T regulatory region in men affected by non-obstructive azoospermic (NOA).
Materials and Methods: This study is conducted among a total number of 200 men, including 100 infertile men affected by NOA and CMA (Complete Maturation Arrest) testis histopathology. The control group was fertile men that at least have one child. All men have normal karyotype and also no microdeletion in AZF (Azoospermia Factor) region. To study the genetic variations, Polymerase Chain Reactions (PCR) for HIST1H1T gene was performed using specific primers that amplified from +208 to -343 of the gene and followed by direct sequencing - so far, 50 samples have been sequenced and the rest of them are being sequenced over time. The statistical analysis was done using Chi Square (P<0.05).
Results: An exonic SNP with rs198844 was detected in heterozygote form in 44% of the sample patients and in homozygote form in 30% of the sample NOA men.
Conclusion: This is the first report on transcription factor binding site of HIST1H1T investigation in male infertility. Our results suggested that the single nucleotide polymorphism (rs198844) can be assumed as a contributing factor for male infertility.
Keywords: H1t, HIST1H1T, Genetic Variation, Spermatogenesis Failure, Male Infertility

P-135: The Evaluation of Relationship between No Call Cell Free DNA Tests and Fetal Fraction, Maternal BMI, CRL and PAPPA MOM

Moshfeghi M[1*], Eftekhari MH[2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Medical Ethics, Tehran University of Medical Sciences, Tehran, Iran
Email: moshfeghimaryam@yahoo.com

Background: Non-invasive prenatal detection of fetal trisomies 21, 18 and 13 has been made possible by examining cell-free DNA (cfDNA) in maternal plasma. This test has some no call. the aim of this study is to examine the possible effects of maternal and fetal characteristics including PAPPA MoM, CRL and BMI on the fetal fraction in Maternal plasma cfDNA at 11–13 weeks' gestation.
Materials and Methods: About 300 cases of positive risk of trisomy 21 in combined test , that desired to refer for cell free test, were entered in our study, we recorded PAPPA mom , CRL, and BMI and then analaysed them. we followed the no call cases and the others until delivery and so trust of euploid baby. we evaluated the relationship between no call and fetal fraction and our parameters in euploid and aneuploid cases.
Results: From 300 cases , 20 cases were no call and needed invasive tests and 3 cases were aneuploid finally, PAPPA MOM<0.4 in no call cases were 60%(n=12), in Tri13 0.3%(n=1), and tri21 0.6%(n=2).PAPPA mom 0.4 -0.6 in 26% (n=80),>0.6 in 66% (n=200) were observed.in all cases of no call fetal fraction in maternal plasma were<3%.and in others were more than 4%.we detected that BMI>30 was seen in 60% (n=12)of no call and in 7.1% (n=280) of reasonable results. No meaningful relationship was detected between CRL and results .we observed meaningful statistical result about low PAPPA mom and BMI>30 in non responded tests and uneuploidy as well (P value<0.05).
Conclusion: The ability to detect trisomy 21, 13, 18 with cfDNA is dependent on fetal fraction that usually should be over 3% which is depend on maternal BMI , PAPPA mom and certainly other parameters, that we did not enter them in our study.
Keywords: Trisomy 21, Trisomy 18, Non-Invasive Prenatal Diagnosis, Fetal Fraction

P-136: The Correlation between Human Sperm Parameters and PPARs Gene Expression

Mousavi M[1*], Alizadeh A[1], Sayahpour FA[3], Esmaeili V[1], Sharbatoghli M[1], Maroufizadeh S[2], Rahimizadeh P[1], Shahverdi AH[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem cells and Developmental Biology, Cell science Research Center, Royan Institute for Stem cell Biology and Technology, ACECR, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: The proxisome proliferated- activated receptors (PPARs) belong to a subfamily of the nuclear receptor superfamily of ligand-inducible transcription factors. The PPARs isotypes (alpha, beta and gamma) play crucial roles in carbohydrate and lipid metabolisms. Although the pivotal roles of these receptors on female fertility have been confirmed, few studies have been done on PPARs on male fertility. We determined several PPARs gene expression in human sperm and the correlation between sperm parameters and these gene expressions.
Materials and Methods: Human semen have been collected, according to the WHO recommended procedure from men (n=80) undergoing semen analysis for couple infertility in Royan institute. The semen parameters were determined by CASA. The human ejaculated spermatozoa mRNA was isolated by RNeasy plus universal Mini Kit Qiagen. PPARα, PPARβ and PPARγ gene expression determined by real-time PCR. Beta actin used as house keeping gene. Data were analyzed using the MIXED procedure of SPSS2.
Results: PPARs gene expression have been seen in human

sperm. The PPARγ gene expression levels had a significant positive correlation with sperm concentration, motility and progressive cells (r= 0.346, r= 0.317, r= 0.300, respectively P<0.01). In addition, there was a significant negative correlation with expression of this gene and percentage of immotile sperms (r= -0.317, P<0.01). PPARα gene expression had a positive significant correlation with sperm motility (r=0.401, P<0.05). PPARβ expression had no significant correlation with sperm parameters.
Conclusion: Our data indicated that several PPAR genes were expressed in human sperm. These genes may affect male fertility which warrants further studies to understand PPARs roles in sperm.
Keywords: PPARs, Sperm, CASA, Sperm Motility

P-137: Expression of Cyclooxygenase mRNA in Testes of Ram by Pomegranate By-Product Consumption

Nikfarjam M[1], Shahverdi AH[2], Ghoreishi SM[3*], Alizadeh AR[2]

1. Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Animal Science, College of Agriculture, Shiraz University, Shiraz, Iran
Email: shahverdi@royaninstitute.org

Background: Recent studies have demonstrated that pomegranate composition have antioxidant and anti-inflammatory properties. Although cyclooxygenase as a key enzyme involved in the inflammatory process, limited information exists on effect of pomegranate on COX (cyclooxygenase) expression in reproductive organs. We investigated the effect of pomegranate peels and /or seeds on COX-1 and COX-2 expression in rams'testes.
Materials and Methods: Twenty-four Iranian rams were randomly divided into three groups and fed either of the three diets for 80 days: control diet (C), diet containing 31% pomegranate seeds (PS), and diet containing 27% pomegranate peels (P) . Experimental groups were offered isoenergitic and isonitrogenous rations. Testicular tissue samples were taken and quantified by real-time RT PCR for mRNA abundance of COX-1 and COX-2. The mRNA abundances were measured relative to the expression of PTGS1 and PTGS2 as reference genes. Data were analyzed using the GLM procedure of SPSS.
Results: COX-1 expression was not significantly affected by experimental diet. Similarly, COX-2 was unaltered by treatments.
Conclusion: In conclusion, the present results showed that inclusion of pomegranate peels and/or seeds may not alter COX-1 as well as COX-2 expression in rams, which warrants further studies.
Keywords: Pomegranate, Cyclooxygenase, Ram Testes

P-138: Expression Analysis of GDF9 Gene in Granulosa Cells of Diminished Ovarian Reserve (DOR) Patients Referred to Royan Institute

Omrizadeh M[1, 2*], Mirfakhraee R[1], Zarei Moradi SH[3], Ghaffari F[1], Mokhtari P[3], Mohseni Meybodi A[3]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: anahitamohseni@gmail.com

Background: Diminished ovarian reserve (DOR) and/or poor ovarian response (POR) is a tough issue encountered during assisted reproductive technology (ART). DOR affects 10% of women seeking fertility treatment. DOR can be a normal process of aging. In other cases DOR occurs at earlier ages, pathological DOR, a multifactorial condition which may be caused by genetic disorders. The growth differentiation factor 9 (GDF9) paracrine factor belonging to the superfamily TGF-β, which is essential for folliculogenesis. The GDF9 participate in the evolution of the primordial follicle to primary follicle and plays an important role in the later stages of follicular development and maturation.
Materials and Methods: This case-control study included 12 women with DOR and 12 controls, which were undergoing *in vitro* fertilization (IVF). DOR patients have been selected by: abnormal ovarian reserve testing may base on decreased antral follicle count (AFC<5), decreased anti müllerian hormone (AMH<0.5-1.1 ng/ml) or elevated levels of follicle stimulating hormone (FSH>10 IU/L on cycle day 2 to 4). Mural granulosa cells were isolated at oocyte retrieval; messenger RNA was extracted and cDNA synthesized and Real-Time PCR was performed for expression assay.
Results: GDF9 gene expression has been demonstrated in all regions of the GCs in both groups by using RT-PCR. Much lower expression of GDF9 gene was detected in all parts of GCs from DOR women compared to controls (P value ≤0.05).
Conclusion: Reduction of GDF9 expressions in follicle fluid with DOR indicates that GDF9 has important role in outbreak of DOR. Decrease of GDF9 in DOR compare to control group that may resulted in abnormal growth and immaturation of follicles and oocytes in DOR patients.
Keywords: Diminished Ovarian Reserve, Growth Differentiation Factor 9 (GDF9), Folliculogenesis, Expression

P-139: Association of Thymic Stromal Lymphopoietin (TSLP) Polymorphism in Different Tissues (Peripheral Blood and Endometrioma) with Endometriosis

Poursadoughian Yaran A[1, 2*], Naji T[1], Aflatoonian R[3], Amirchaghmaghi E[3, 4], Ramazanali F[3], Shahhoseini M[2], Afsharian P[2]

1. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: pafshar@royaninstitute.org

Background: Endometriosis is a disease determined by the inflammatory peritoneal environment characterized by the growth of endometrium-like tissues in ectopic region. Despite the decades of research, the pathogenesis of endometriosis remains elucidated and multiple theories exist regarding its etiology. Indeed, an immunological cause has been hypothesized, owing to the elevated levels of immune mediators like activated macrophages, cytokines, T and B cells. Thymic Stromal Lymphopoietin (TSLP) is a novel cytokine which maybe has an important role to aptitude of endometriosis. It is an IL-2 like type 1 inflammatory cytokine that triggers differentiation on Naïve CD4 Tcell to T helper 2 leading the immune response. TSLP has 23 polymorphisms that one of them is located in promoter C-847T (rs3806933) which constructs a binding motif for transcription factor activating protein 1 (AP-1)subsequently increases expression of TSLP. The objective of current study is detecting the genotype frequency of TSLP promoter polymorphism in patients with endometriosis.
Materials and Methods: In this case-control study, we studied 100 women with endometriosis (diagnosed by laparoscopy and confirmed with histopathological test) who referred to Royan institute and 100 fertile women with one child by natural conception as a control group. DNA was extracted from whole blood and 28 endometriotic tissue from same patients. Polymerase chains reactions (PCR) and direct Sanger sequencing were performed. The statistical analysis was done by Chi Square ($P<0.05$).
Results: The genotype frequency of CC, CT and TT in peripheral blood sample of patients group were observed 9%, 61% and 30% respectively, whereas they were 16%, 44% and 40% in control group ($P=0.046$). We evaluated this genotypes distribution in 28 blood sample of patients compared to ectopic tissue. In these patients the CC genotype wasn't detected, however, CT and TT genotypes were identified 78.5% and 21.5% in ectopic tissue, respectively. Also, the frequency of CT and TT in blood of selected patients were 64.5% and 35.5% respectively, however the alteration was not significantly different.
Conclusion: The promoter SNP (rs3806933) of TSLP is very common in inflammatory disorders and it seems the assessment of this polymorphism in ectopic tissue could create a more accurate view for this influence in etiology of endometriosis. According to our results, this SNP was significantly related to susceptibility of endometriosis. Also the frequency of C-847T genotypes are altered between ectopic tissue and peripheral blood which can be caused by mosaicism, however a larger sample size study is essential.
Keywords: Endometriosis, Polymorphism, Thymic Stromal Lymphopoietin, TSLP, Tissue Mosaicism

P-140: Cancer Detection Using Circulating microRNAs in Peripheral Blood Improved Couple's Fertility

Sadeghi H[1, 2*], Sharifi Zarchi A[3], Kamal A[4], Shayesteh Pour B[1, 2], Gourabi H[2], Keller A[5], Totonchi M[1, 3]

1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Clinical Bioinformatics, Saarland University, Saarbr¨ ucken, Germany
4. Department of Genetics, University of Science and Culture, ACECR, Tehran, Iran
5. Department of Mathematical Sciences, Sharif University of Technology, Tehran, Iran
Email: m.totonchi@royaninstitute.org

Background: The use of personalized medicine to advance prevention and cure of disease is potentially possible. The main step to success in this filed depends on having diagnostic tests that identify patients who can benefit from therapies. The chemotherapy regimens used for the treatment commonly affect fertility and cause premature ovarian failure (POF). microRNAs (miRNAs) are non-coding RNAs that regulate many cellular processes including tumorigenesis. Circulating miRNAs are known as less invasive markers in many malignancies. Recent studies have shown that some specific miRNAs are deregulated in blood of early stage cancer patients compared to healthy controls. In this study, we aim to design subsets of circulating miRNAs can detect each type of cancer from unaffected controls and other types of cancers with high accuracy.
Materials and Methods: We used miRNA expression profiles from the cancer genome atlas and analyzed 6104 next-generation sequencing data related to 14 different types of cancer tissues encompassing 5493 cancer samples and 611 healthy controls. We were using feature selection algorithm and support vector machine with 10 fold cross validation as machine learning method for improving detection accuracy.
Results: By focusing on five miRNAs, we could separate all cancer samples from all normal samples with 97% accuracy. We obtained subsets with maximum 5 members and also acceptable accuracy for each cancer type. The highest accuracy received for thyroid carcinoma (98%) and kidney renal clear cell carcinoma (97%) with subset of three and two miRNAs, respectively. We also could classify samples in 3 classes (breast invasive carcinoma, normal breast tissue and all other normal and cancer tissues) just with 3 miRNAs.
Conclusion: Using these bioinformatics approach we identified various subsets of miRNAs that could distinguish every type of cancer from unaffected controls in early stages and preserved fertility. These subsets have potential to be evaluated in blood samples of each cancer type.
Keywords: Fertility, Early Detection, Circulating microRNA, Biomarker, Bioinformatics

P-141: Study of 4G/5G Mutations Prevalence in Plasminogen Activator Inhibitor-1 Gene in Iranian Women with Ectopic Pregnancy

\
Sadeghi Z[1*], Ebrahimi A[2], Seyedena SY[1], Davari Tanha F[3]

1. Cellular and Molecular Research Center, Institute of Endocrinology and Metabolism, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Obstetrics and Gynecology, Vali-e-asr Reproductive Health Center, Tehran University of Medical Science, Tehran, Iran
3. Department of Cellular and Molecular, Faculty of Biology Sciences, Islamic Azad University of Tehran-North, Tehran, Iran
Email: z_sadeghi@email.com

Background: Ectopic pregnancy (EP) is a type of pregnancy occurring at extra uterine sites and the most common site being the fallopian tubes. This disorder is one of the reasons for maternal mortality in first trimester of pregnancy and commonly causes abortions. Many problems are caused by this disorder

and bleeding occurs in majority of the cases. On the other hand, plasminogen activator inhibitor-1 (PAI-1) gene is one of factors involved in unsuccessful pregnancies and 4G/5G polymorphism is the most important change seen in this gene. So, it's important to study the prevalence of this alteration in this gene in women with EP.
Materials and Methods: In this Case-Control study, we have chosen 100 Iranian women with at least one time history of Ectopic Pregnancy as Case group, and 101 Iranian women with the normal pregnancy without any history of EP as Control group. After blood sampling, all subjects were genotyped for this polymorphism, using ARMS PCR (Amplification Refractory Mutation System Polymerase chain reaction) and data were analyzed by statistical analysis. P value of<0.05 was considered as significant.
Results: In this study 4G allele with 70.79% prevalence and 5G allele with 63.5% are most common alleles in the Control and Case groups respectively. Prevalence of 4G/4G and 4G/5G genotypes in the Control group are 54.5% and 32.7%, respectively and Prevalence of 4G/5G and 5G/5G genotypes in the Case group are 51% and 38%, respectively. An Armitage test found P<0.05 for both alleles and showed that 4G allele (P=1.524e-10 ; OR= 0.262) has decreasing effect and 5G allele (P=1.524e-10 ; OR= 3.822) has increasing effect in EP.
Conclusion: 5G allele and 4G/5G and 5G/5G genotypes have increasing effect, 4G allele and 4G/4G genotype have decreasing effect in EP. So we could consider 5G allele as a risk factor of ectopic pregnancy in this study.
Keywords: Ectopic Pregnancy, PAI-1 4G/4G

P-142: Genetic Variant(s) Analysis of TEX12 Gene in Couples with Recurrent Spontaneous Abortion (RSA)

Shariati Seyasar M[1, 2*], Mojarrad M[3], Mohseni Meybodi A[3], Hassanzadeh Nazarabadi M[3]

1. Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Molecular Genetic, Faculty of Basic Sciences and AdvancedTechnologies in Biology, University of Science and Culture, Tehran, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, , Tehran, Iran
Email: nazarabadim@mums.ac.ir

Background: Recurrent spontaneous abortion (RSA) is a complex problem that genetic and environmental factors are involved on it. One of the causes of spontaneous abortion may be genetic disorders, particularly chromosomal aneuploidy. The formation of the Synaptonemal complex structure role is one of the critical factors in relation to correct chromosomal segregation during meiosis. However any disorder in this structure can be cause of chromosomal aneuploidy. Testis-expressed gene 12 (TEX12) is a germ cell-specific gene that is located on the chromosome 11 (11q22) in humans. Its protein product specifically localizes to the central element structure of synaptonemal complex.TEX12 is exclusively expressed in mice and humans germ cell. The absence of TEX12 results in a disrupted central element and only partial synapsis of the meiotic chromosomes, which could have consequences for the progression of meiotic recombination and lead to infertility in mice. The aim of this study was to determine the frequency of genetic variation of TEX12 gene in couples with the history of recurrent spontaneous abortion with normal karyotype that were referred to the Royan Institute.
Materials and Methods: Fifty couples with a history of RSA (more than two consecutive pregnancy losses) of unknown cause were enrolled in this study. Furthermore, as well as fifty couples without the history of RSA and at least have one healthy child were be selected as a control. Genomic DNA was extracted from peripheral-blood samples. Primers for all of the encoding exons were designed. High Resolution Melting (HRM) was performed under standard conditions. Suspected cases will be further analyzed by Sanger sequencing.
Results: Our data will be prepared until presentation time.
Conclusion: Mutations of TEX12 may be involved in recurrent spontaneous abortion.
Keywords: TEX12, Recurrent Spontaneous Abortion, Synaptonemal Complex, Chromosomal Aneuploidy

P-143: The Investigation of WT1 Gene Exon 9 Mutations in Azoospermic Infertile Iranian Men with Undescended Testis

Sharifi N[1, 2], Mohseni Meybodi A[2*], Sabbaghian M[3], Farrahi F[3]

1. Department of Molecular Genetic, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: anahitamohseni@gmail.com

Background: The incidence of infertility in men is high around the world, so investigating the factors is important in infertile patients. Genetic disorders, azoospermia, hormonal dysfunctions or reproductive system diseases are the main causes of male infertility. For instance, cryptorchidism or undescended testis (UDT) is a urogenital disease that has infertility consequence. So it is beneficial to detect the mutations of genes in these kind of diseases. One of the substantial genes in reproductive system is Wilms Tumor 1 (WT1). It is proved that, WT1 is involved in development of reproductive system, hormonal synthesis, urology and renal diseases. WT1 is located on chromosome 11 in the region of 11p13 and encodes four isoforms. It comprises 10 exons and has an alternative splice donor site which is located at the end of exon 9 and it can insert three lysine, threonine, and serine amino acids (abbreviated as +KTS/- KTS) between the third and fourth zinc fingers. The existence of + KTS /-KTS in proteins level play different rolls. So due to the correlation between WT1, UDT, and infertility and hormonal dysfunctions, it is noteworthy to investigate the possible mutations in exon 9 of WT1 gene in infertile azoospermic patients with UDT.
Materials and Methods: A case-control study was performed in this research. 60 infertile azoospermic Iranian patients with UDT as a case group and 60 fertile men who referred to Royan institute for sex selection as a control group were enrolled in our research. All participants were selected with normal karyotypes and no AZF microdeletions. Also, UDT patients were included after clinical examinations, hormonal tests (increased of follicle stimulating hormone (FSH), luteinizing hormone (LH) and decreased of Testosterone), and semen analysis and were excluded with renal diseases or Wilms tumor. Polymerase chain reactions (PCR) were used for Exon 9 of WT1 gene and followed

by direct Sanger sequencing. 40 samples have been sequenced and the rest of them are being sequenced over time. Finally, Chi square method was done for statistical analysis (P<0.05).
Results: No significant mutations were observed in exon 9 of WT1 gene. Just an exonic synonymous SNP with rs28941778 was detected in heterozygote form of the control group.
Conclusion: This is the first investigation of exon 9 in WT1 in the group of infertile UDT Iranian patients. Our results suggests that the alternative splice donor site in exon 9 cannot be related to male infertility, UDT or in azoospermia.
Keywords: WT1 Gene, Male Infertility, Undescended Testis, Azoospermic

P-144: Aberrant Expression of Homeobox (Hox) 6 and 8 Genes in Cumulus Cells of Infertile Women with Poly Cystic Ovary Syndrome in Royan Institute

Tabatabaei SZ[1, 2], Eftekhari Yazdi P[3], Hafezi M[4], Zarei Moradi Sh[2], Shahhoseini M[1, 2]

1. Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute, ACECR, Tehran, Iran
3. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute, ACECR, Tehran, Iran
4. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute, ACECR, Tehran, Iran
Email: m.shahhoseini@royaninstitute.org

Background: Polycystic ovary syndrome (PCOS) is the most common endocrinal disorder that affects anovulatory infertile women. Numerous factors such as environment, metabolism, hormones and genetic are prone to cause PCOS. Of genes that have altered expression in human reproductive system disorder are HOX family genes. Homeobox genes are master genes that act at the top of genetic hierarchies regulating aspects of proliferation, differentiation, organogenesis, morphogenesis, adhesion and migration. Cumulus oophorus is a cluster of cells, called cumulus cells that surround the oocyte both in the ovarian follicle and after ovulation. Follicular development, oocyte maturation, ovulation and fertilization are the momentous role of CCs.
Materials and Methods: This study was designed to compare 20 PCOS patients as a case and 20 fertile women with male infertility problems referred to the Royan Institute to get ICSI under GnRH antagonist protocol as a control population. Cumulus cells were collected from PCOS patients and fertile. Informed consents were obtained from the participants. Thirty six hours after hCG injection, ovaries were punctured and cumulus oocyte complexes were dissected. Total RNA was extracted from CCs and cDNA was synthetized by whole transcriptome amplification kit. Quantitative Real-time PCR was performed by use of specific primer for HOXC6 and HOXC8 genes.
Results: Obtained data showed significant increase of HOXC6 expression (P<0.05) in PCOS patients vs. control group. On the other side, there was a significant decrease in expression of HOXC8 (P<0.05) in PCOS patients vs. control group.
Conclusion: Current study almost confirms significant correlation between altered expression of HOX family genes and PCOS disorder and provides new insights to understand the pathogenesis of PCOS.
Keywords: HOX Genes, Infertility, PCOS, Cumulus Cells

P-145: Genetic and Molecular Study of Toll-like Receptor 3 (TLR3) in Endometriosis

Zamani Almasi M[1, 2*], Moini A[1], Ramazanali F[1], Hosseini E[3], Shahhoseini M[2], Afsharian P[2], Aflatoonian R[1]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Obstetrics and Gynecology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
Email: R.Aflatoonian@gmail.com

Background: Endometriosis is an estrogen-dependent, common and benign disease and is characterized by the growth of endometrial tissue that is found primarily in the peritoneum, ovaries and rectovaginal septum. Recently, endometriosis has been alternatively described as an immune, genetic and hormonal disorder. Toll-like receptor 3 (TLR3) is a member of the TLR family which plays a critical role in innate immunity and involves in the processes of cell proliferation, survival, apoptosis, angiogenesis, tissue remodeling and repair through directly recognizing exogenous and endogenous ligands and leads to expression of some inflammatory genes such as Interleukin-6 (IL-6) and IL-8 by NFkB pathway. TLRs should play important role in pathogenesis of endometriosis, therefore in the current investigation we try to find out TLR3 relation with this disorder.
Materials and Methods: A case-control study along 2012 - 2014 was conducted on 83 endometriosis patients whom had been confirmed by laparoscopic surgery and 93 healthy controls that had no history of inflammatory disorders or using any related drugs. All women taking part in this study were between 20-40 years old. After DNA extraction from peripheral blood samples, Exon4 of TLR3 gene were amplified in two overlapped fragments by polymerase chain reaction (PCR) and products were analyzed by sequencing to determined allele and genotype frequencies. Then, RNA was extracted from tissue samples to analysis of TLR3, TICAM1, IL-6, IL-8 and NFkB gene expression by Quantitative Real-time PCR.
Results: Expression of all five genes was detected in both group but the expression levels were higher in endometriosis patients. The two observed polymorphisms in exon 4 of TLR3 which play a critical role in its signaling pathway, rs3775291 (Missense, CTC → TTC, Leu412Phe) and rs3775290 (Synonymous, TTC → TTT, Phe459=), showed no significant relationship between them and occurrence of endometriosis, although the frequency of C allele (rs3775291) and T allele (rs3775290) was more in the patient.
Conclusion: Inflammation is an essential element in tumorigenesis. These results indicate that TLR3 can trigger an inflammatory response and cell survival by modifying some pro/anti-inflammatory factors in the tumor micro-environment. So TLR3 are critical immunomodulators that may play an important role in the development of gynecologic disorders and can indirectly contribute to tumor progression. Although, TLR3 polymorphism in exon 4 is not effective in the pathogenesis of endometriosis, but other TLR3 mutations which affect TLR3 expression or function may involve in this disorder. Further study should be directed to investigate genetic and epigenetic changes in more samples and in the promoter region.
Keywords: Toll-Like Receptor 3, Endometriosis, Polymorphism, Inflammation, Interleukin

Authors Index

August - 1 Septembe, 2017, Terhran, Iran)

Abstracts of

Royan International Twin Congress

12th Seminar on Nursing and Midwifery
30 August-1 September 2017

Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speakers

I_{nm}-1: Genetic Aspects of Recurrent Abortions

Almadani N

Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email:navidalmadani@yahoo.com

Spontaneous abortion is the most common complication of pregnancy. Approximately 70% of human conceptions fail to achieve viability and almost 50% of all pregnancies ending in abortion before the clinical recognition of the presence of embryonal heart activity.
Recurrent miscarriage is important problem in reproductive health and its occurrence rate is 1%.
The cause of a significant portion (~ 50 %) of the miscarriages cannot be discovered, with this, the most important causes may be listed as; Hormonal dysfunctions, Anatomical anomalies , Auto-immune diseases, …..but Genetic problems take place the major reasons especially in the first trimester for example: chromosomal aneuploidies in sperm and egg related with parents that carry balanced chromosomal translocations (5%) or resulted from maternal meiosis problems or post-zygotic mosaicism. Genetic disease in parents and fetus may induce abortion for example: thrombophlia (mutation in Leiden or MTHFR gene, PT G20210A, …), Autosomal recessive disease (SLOS, Arthrogryposis, …), Autosomal dominant disease (mytonic dystrophy, Achondroplasia, …), X-Linked disease (Rett syndrome, Aicardia syndrome, …).
Recently the microarray and NGS technology have a serious role for detection etiology of recurrent miscarriage.
Knowledge of the genetic background of miscarriage is important for prognosis as well as the potential planning of preimplantation or prenatal diagnosis in subsequent pregnancies.

I_{nm}-2: New Molecular Aspects of Recurrent Spontaneous Abortion

Amirchaghmaghi E

1.Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: amirchaghmaghi_e@yahoo.com

Recurrent spontaneous abortion (RSA) also known as recurrent miscarriage, habitual abortion or recurrent pregnancy loss (RPL) is usually defined as three or more consecutive pregnancy losses prior to 20 gestational weeks. Many etiological factors have been considered as cause of RSA including chromosome abnormalities and other genetic factors, uterine anatomical defects, endocrine diseases, thrombotic and immunologic factors. Nevertheless, the cause of RSA remains unknown in around half of the patients despite ex tensive workup, and thus termed unexplained RSA (URSA).
Several studies suggested that alterations in different molecules, cytokines, growth factors and immune cells at fetomaternal interface may be involved in pathogenesis of RSA. In this presentation, some of important molecular and cellular aspects of RSA will be discussed.
Keywords: Recurrent Spontaneous Abortion, Cytokines, Growth Factors, Immune Cells

I_{nm}-3: Late ART Outcomes

Ashrafi M

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: dr.mahnaz.ashrafi@gmail.com

Assisted reproductive technologies (ART) are widely used in fertility treatment and involve the manipulation of both eggs and sperm in the laboratory. Since the first successful IVF procedure in 1978, IVF has become a common procedure with a record of more than 5 million births. It is estimated that over 200, 000 children are annually born around the world by ART. During this time there have been rapid advances in ART., the effectiveness of IVF has progressed by introduction of embryo cryopreservation and later the more invasive 'technique' of intracytoplasmic sperm injection (ICSI). Preimplantation genetic diagnosis (PGD) is an evolving technique that refers to the genetic profiling of embryos prior to implantation; however, this procedure involves significant embryo manipulation. Available data show that singletons born after ART are at a higher risk of pregnancy complications compared with spontaneous conception; this may be related to the different factors including: background biology of sub-fertile couples, multiple pregnancy, related factor to fertility treatment procedure and embryo endometrium interface. Multiple pregnancy is the most significant complication of ART and is the most powerful predictive factor for adverse maternal, obstetrical, and perinatal outcomes. Ideally, this risk should be discussed with the patient before pregnancy, and couples with good prognosis for success should be counseled concerning the benefits of single embryo transfer (eSET) in reducing the risk of multiples while maintaining the cumulative success rate of the IVF programme. Frozen embryo transfer (FET) is the most common way to reduce the risk of OHSS by avoiding fresh transfer, and singleton pregnancy after FET seems to have a better perinatal outcome than fresh embryo transfer partly due to the embryo selection process.
Although several million children have been born by using ART treatments, but limited documents are present regarding the longer-term health and development outcomes for these children. It has been thought that ART procedure may lead to long term adverse consequences, in addition to the documented adverse perinatal outcome and increased risk of congenital abnormalities in the children resulting from ART treatment. These adverse outcomes encompass respiratory and allergic disorder, endocrine disorders, ophthalmological and auditory disorders, growth and pubertal development, metabolic and cardiovascular effects and risk of cancer. Although there are considerable researches on these effects, but further studies with a long term follow-up are required for a definitive statement or accurate conclusion.

I_{nm}-4: New Embryology Laboratory Strategies for Recurrent Implantation Failure Patients

Eftekhari Yazdi P

Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: eftekhari@royaninstitute.org

Failure of conception despite the repeated transfer of apparently good quality embryos is a significant clinical problem in in vitro fertilization (IVF) practice. The definition of recurrent treatment failure varies but usually includes a number of completed IVF–embryo transfer (ET) cycles and /or the cumulative number of embryos transferred during the unsuccessful treatments. There is considerable interest in the potential causes of recurrent implantation failure and in strategies that may improve implantation through changes in clinical and embryology practice. Even with the transfer of morphologically 'good quality' embryos, their implantation potential may be reduced due to inherent genetic sperm, oocyte or embryo defects. For nearly 40 years, morphology (assessed at a few discrete time intervals) has been the sole means available to the clinical embryologist to gauge the development of the human embryo in vitro. Although several elegant grading systems have been developed to assist in the application of such information to help in embryo selection for transfer, the analysis of morphology alone cannot account for the physiology or karyotype of the embryo. With the advent of commercially available, reliable and effective time-lapse microscopy systems, we are now in a position to assess the embryo at almost unlimited time points. Consequently we are learning more about the impact of specific cleavage patterns, such as direct cleavage to the 3-cell stage (which reflects a compromised and/or aneuploid embryo), and the significance of timings associated with key developmental events. Together with the analysis of embryo metabolism, which not only appears to be associated with embryo viability but has recently been shown to be related to morphokinetic data, we are entering an era where the quantification of viability prior to transfer is looking more promising than ever. Concurrently, we have witnessed rapid developments in molecular techniques to accurately determine the chromosomal complement of the embryo, with a growing move towards trophectoderm biopsy at the blastocyst stage. Hence, we are now in a much stronger position to identify euploid embryos prior to transfer, thereby not only increasing implantation and pregnancy rates but also significantly reducing the time to pregnancy for patients, decreasing patient dropout rates from their clinical treatment.

I_{nm}-5: Communication Skills among Clinical Staff

Ezabadi Z[1, 2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: z_ezabadi@yahoo.com

One of the most vital essentials of human interaction is the ability to communicate, particularly in high-intensity settings such as health care to building effective collaborative relationship procedures as a key role in creating a trustworthy doctor-patient relationship which is a prerequisite for therapeutic success as well as fulfilling the mission and purpose of the organization.
Materials and Methods: A narrative review of all the relevant English-language articles known to the author was conducted.
Results: The quality of care has a direct influence on the quality and safety of patient satisfaction improved by well recognition and understanding of their disease and the treatment available, care management, caring relationships with patients, supporting their physical, mental, and spiritual requirements, and the accurate information to facilitate discussions and enhance team function. Obstacles to effective communication by three basic components-verbal, non-verbal and para-verbal, encompass such elements as insufficient time, hierarchies, defensiveness, resistance to change, horizontal or vertical distrust, cost concerns varying communication styles, distraction, fatigue, and workload barriers that could obstruct implementation of the Principles are more intimidating than they are real. The most important one is lack of insight due to inadequate knowledge and training in interaction skills.
Conclusion: Good communication skills among the teams of providers not only helps in therapeutic success by providing holistic care to the patient but also leads to satisfaction among patients, families, and health care team members that no single provider can contribute.
Keywords: Communication Skills, Healthcare Professionals

I_{nm}-6: Recurrent Abortion, Male Aspects

Farrahi F

Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: farrahif@yahoo.com

Reproductive failure is a common complication in early pregnancy, with up to two-thirds of all fertilized oocytes not producing live births. Male gamete contributes one-half of the genomic content to the embryo. ESHRE guidelines define recurrent miscarriage (RM) as three or more consecutive pregnancy losses before 22 weeks of gestation. A chromosomal abnormality in one partner is found in 3 to 6% of RM couples, which is ten times higher than the background population. There is a documented link between sperm DNA damage and RM. Gene mutations, paternal age, measuring lipid peroxidation and antioxidant capacity of seminal plasma in addition to conventional sperm parameters, recommended in couples with recurrent embryo losses.

I_{nm}-7: Holistic Nursing for Patients Undergoing Infertility Treatment

Habr GN

Clemenceau Medical Centre, IVF/The Women Clinic Nurse Manager, Beirut, Lebanon
Email: grace.habr@cmc.com.lb

Due to high prevalence of infertility, increasing demand for infertility treatment, and provision of high quality of fertility care, it is necessary for healthcare professionals especially nurses/ midwives to explore infertile couples' expectations and needs. Identification of these needs can be a prerequisite to plan the effective supportive interventions.
It is vital for infertility nurses to understand the psychological impact of infertility on patients, in terms of knowing what and not to say to them in an effort to comfort, as well as how best to support patients through their journey from diagnosis through

treatment.
Intensive Midwifery/Nursing care has been shown to not only increase pregnancy rates, but to lower distress; therefore, stress reduction skills are a valuable tool to use with these patients.
In addition, the role of the infertility nurse is continually expanding and changing to meet the demands of couples undergoing assisted reproduction; Nurses and midwives need to undertake research into their practice in fertility care, that is, caring for infertile people as they undergo assisted reproductive technologies and, to this end, suggests sources of research funding.
In this session, we will discuss the responsibilities of infertility nurses during ovulation induction programs in fertility clinic. The extended role of infertility nurse allows more continuity of care and better understanding of patient's needs and results in the involvement of fewer people in the overall care. Also we will discuss the quality improvement of the healthcare services by development of patient centered approaches and couple-based interventions so as to reduce infertile patients' psychological stress induced by fertility problems. The IVF nurse/midwife is an untapped resource for recruiting and retaining IVF patients.
Finally, it is not uncommon for nurses to experience signs of stress and tension from working with this intense and needy patient population; recognizing these symptoms in oneself and learning how to nurture oneself, is key

I_{nm}-8: Empty Arms, Broken Heart

Habr GN

Clemenceau Medical Centre, IVF/The Women Clinic Nurse Manager, Beirut, Lebanon
Email: grace.habr@cmc.com.lb

Stress and anxiety are natural responses when we are frightened or threatened in any way.
Fertility problems and IVF treatment can represent a threat to patient's future dreams, self-esteem, intimate relationships with their partner, sense of normality or other relationships.
Facing infertility and undergoing fertility treatment can create emotional turmoil in couple's lives. It is essential for fertility therapy providers to assess the coping and communication strategies of couples before treatment in order to provide appropriate support.
Fertility treatment involves various diagnostic procedures, medical treatments and repeated interventions, which can be successful, but often are not. This long-lasting process creates specific emotional challenges with significant impact on couple's intimate lives. It is, therefore, essential that a couple's ability to cope and the strength of their relationship should be considered and assessed prior to treatment, particularly during IVF.
In this session we will discuss the different symptoms of anxiety in IVF patients, identify various coping strategies to help decrease anxiety in these patient population and explain the role of healthcare professionals to help patients reduce anxiety.
Psychological problems concurrent with fertility problems has been the focus of substantial scientific inquiry. However, researchers have largely overlooked psychological resilience within this patient's population. Research has shown that Resilience is negatively associated with infertility-specific and general distress. Engagement in action-focused coping skills was positively correlated with resilience. In this session, the relationship between building resilience and coping strategies to reduce anxiety in this population will be discussed.

I_{nm}-9: Teamwork in the Healthcare System

Hosseini SJ

Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: jhosseinee@gmail.com

Health is one of the most important human concerns and the health system has an increasing role in protecting and improving the health of people in different aspects. Providing qualified health care requires a large number of specialties in a highly complex environment with many effective factors that interact effectively and constructively.
In fact, providing health care as a team effort is inextricable and no one can complete the healthcare delivery chain alone. The use of teams has increased significantly in health service providers. A team attitude to health problems leads to improving health care delivery (quality, equity, access, efficiency, etc.). The concept of a team in the health system is associated with supra-professional values such as community perception of social justice requirements, budget priorities has been entangled for health services, and professional values (such as power and discretion).
Teamwork in the health care environment is defined as follows: "A dynamic process involving two or more health care professionals with a background of common health skills, goals and activities, and doing physical and spiritual actions in assessing, planning or evaluation health care through coherent collaboration, open communication, joint decision making and value added production for patient, staff and organization.
According to the World Health Organization, once a health care team has the most success, that : the goal, appropriate communication, coordination, conflict resolution mechanisms, and the committed participation of each member.
A review of numerous studies conducted in the field of teamwork showed that factors such as: structural and process factors (size, variety of jobs, communications, etc.), team cohesion and integrity, the existence of common transparent goals, the existence of audits within the team, regular team feedback Individual characteristics and employee commitment, intra team communication, and the opportunity to develop creative work methods inside the team, respect and mutual trust, transparent leadership, job satisfaction, and other factors can facilitate team and team performance. It is clear that the lack of these agents and factors will create barriers to the advancement of organizational goals and teamwork.
Promoting and using the concepts of teamwork in the health system can have many benefits to that health system. To promote these concepts, using successful experiences of managers and similar environments in the same cultural context can help the organization and its members achieve their goals.
Keywords: Teamwork, Health System, Health Care Provider Organizations

I_{nm}-10: The Effect of Sperm DNA Fragmentation on Miscarriage Rates

Karimian L*, Hassani F

Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: L_karimian@yahoo.com

Sperm DNA Fragmentation has been extensively studied for more than a decade and may associated with reduced fertilization rates, embryo quality, pregnancy rates and increased miscarriage rates. Various methods exist to test sperm DNA fragmentation such as the sperm chromatin structure assay (SCSA), the sperm chromatin dispersion (SCD) test, the terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay and the single cell gel electrophoresis (Comet) assay.
Recurrent pregnancy loss is an important reproductive health issue, affecting 2-5% of couples. Common established causes include uterine anomalies, antiphospholipid syndrome, hormonal and metabolic disorders, and cytogenetic abnormalities. Other etiologies have been proposed but are still considered controversial, such as chronic endometritis, inherited thrombophilias, luteal phase deficiency, and high sperm DNA fragmentation levels.
Sperm DNA fragmentation is prevalent among infertile men and is known to influence natural reproduction. However, in different studies the impact of sperm DNA damage on assisted reproduction outcomes remains controversial.

I_{nm}-11: Midwifery Care in The Difficulty ART Patients Management

Mirghavamdin NS

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: Naeimesadat@yahoo.com

Various studies have shown the subfertility can be experienced as traumatic life events, causing a sense of loss, failure, extreme exclusion and other social, economic and psychological consequences. Infertility treatments may require significant physical, psychological and financial Investment from the woman and her partner.The journey to become pregnant through ARTS can be difficult and prolonged for the couple.
In addition, women who become pregnant following infertility treatments maybe more anxious because of their heightened fear of losing the pregnancy and the increased risks associated with an IVF pregnancy.
A woman's anxiety may be exacerbated if she is under care of health care provider who does not appear to fully understand the uncertainties and difficulties they have experienced having conceived through ARTs.
In this situation, midwives need to maintain a balance between : acknowledge the emotional investment that a couple take place in the pregnancy along with their anxiety and concerns, with the goal of keeping pregnancy as normal as possible and building the woman's confidence and self-esteem.
In order to providing safe and effective care to such women, midwives need to have a good understanding of infertility and ARTs so that their care wouldbe "based upon the integration of knowledge derived from the arts and sciences".
Midwives must be prepared, aware, present and supportive in keeping hope, healing, spiritual well-being, psychological adaption, life satisfaction and a state of well-being, within a multidisciplinary healthcare team. Equally essential is to be respectful of each human singularity as well as their personal values, religion and beliefs, in the context of living with infertility.

I_{nm}-12: Recurrent Abortion work-up

Moini A.

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Gynecology and Obstetrics, Arash women's Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Vali-e-Asr Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Email: a_moini@royaninstitute.org

The vast majority of all early pregnancy losses result from chromosomal abnormalities arising in the egg, the sperm, or during early embryonic development and are random events. Even repeated miscarriages can occur by chance alone, but at least some affected couples have a predisposing factor. Among all the factors that have been implicated, the only undisputed causes of recurrent pregnancy loss are genetic (balanced chromosomal translocation in either partner, maternal age-related increase in prevalence of aneuploidy oocytes), anatomic (congenital and acquired uterine abnormalities), or immunologic (the thrombotic complications of antiphospholipid syndrome). Alloimmunopathology, inherited thrombophilias (Factor V Leiden and others), endocrinopathies (thyroid disorders, diabetes, luteal phase deficiency), infections (genital mycoplasmas), and environmental exposures (smoking, heavy alcohol or caffeine consumption) have been implicated but are not established causes of recurrent pregnancy loss. Even after a comprehensive evaluation, recurrent pregnancy loss remains unexplained in well more than half of affected couples. For all couples who have suffered recurrent pregnancy loss, education can provide important perspective; most couples welcome the offer of evaluation to identify any predisposing factor. When a likely cause can be defined, specific counseling and treatment can improve the prognosis for a successful pregnancy. When no specific cause can be found, reassurance and encouragement are no less valuable.

I_{nm}-13: Recurrent Abortion and Poor Pregnancy Outcome

Mollaahmadi F.

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: f_ahmadi5263@yahoo.com

Background: Despite years of research, recurrent pregnancy loss (RPL), recurrent miscarriage, or habitual abortion, defined as the occurrence of three or more consecutive losses of clinically recognized pregnancies prior to the 20th week of gestation, continues to pose a medical challenge.
Materials and Methods: Informative review of possible causes and management of habitual pregnancy loss and infertility.
Results: Early pregnancy loss, is a relatively common occurrence in 15–25% of pregnancies, and increasing in prevalence with maternal age. Indeed, the risk is between 9 and 12% in women aged<35 years, but increases to 50% in women aged>40. It is acceptable to start a workup following two repeated losses, especially in women aged>35 years with a comprehensive gynecologic history for couple, families and information about previous pregnancies. Both partners should also be questioned about the modifiable lifestyle factors, such as Obesity, defined as a body mass index>30 kg/m,

smoking, excessive caffeine consumption (>300 mg/day, or the equivalent of two cups), excessive alcohol intake, and nutritional habits. Regardless of the cause, a full workup ordered following the initial visit to identify treatable causes including uterine abnormalities, APS, endocrine diseases, and balanced translocations to support, can help most couples achieve a successful live birth. It is an established fact that the presence of appreciable amount intake of micronutrients, such as folic acid and zinc, magnesium, iron and vitamins before and during the conception to be essential for normal embryogenesis. The women administered antioxidants in studies exposed enhancement in the birth rate, chances of outcome, reduction in still birth, maternal mortality, and gestational duration. Therapy should be concentrating any curable etiology, and may include ICSI with PGD, use of donation or surrogacy, and surgical correction of anatomic abnormalities, correction of endocrine disorders, sympathetic counseling and anticoagulation or folic acid supplementation. It is important to remember that most women with RPL have a good prognosis for eventually having a successful pregnancy, even when a definitive diagnosis is not made and no treatment initiated.

Conclusion: RPL is an important reproductive health issue as a profound personal tragedy require empathy and understanding as early pregnancy loss is an emotionally traumatic experience various etiologies have been identified over the years and successful therapeutic strategies implemented.

Keywords: Recurrent Pregnancy Loss, Poor Pregnancy Outcome, Workup

I_{nm}-14: The Ethics, Moral, Legal and Social Issues in Preconception Sex Selection

Omani Samani, R

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: r.samani@gmail.com

I_{nm}-15: The Relationship between Teamwork and Organization Trust

Sabeti Sh

Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: Sh.sabeti73@gmail.com

The doctor patient relationship is a keystone of care system. This is the one of most important issue in health care system such as infertility center. To be satisfied with physician is a main factor for people to begin treatment, stay and obey the rules. This is a skill that every doctor should know how to relate more effectively and efficiently. The medical interview is the major step of health care, when a patient does not trust or like the doctor will not disclose information. Efficient relationship determines the quality of information. Respect, caring, empathy, self disclosure positive regard, congruence, understanding, allows patients to express and reflect their feeling are the main items to form the relationship. To create a good atmosphere of caring organize a patient -centered culture.

I_{nm}-16: Respect and Empathy: Two Communication Skills of The Physician with The Patient

Shojaei AA.

Research Center for Medical Ethics and History, Faculty of Medical Ethics, Tehran University of Medical Sciences, Tehran, Iran
Email: dramirahmadsh@yahoo.com

Beneficial physician-patient communication , is a centric function in medicine and is achieved from the talent and skill of doctors. So many patients dissatisfaction and complaints are due to bad relationship with doctors. communication skills are various and a lot, but empathy and respect are more important of them in relationship of patient- physician. So we are going to describe and explain and justified them regarding to nowadays social situation.

Keywords: Physician-Patient Communication, Empathy, Respect

I_{nm}-17: Stem Cells as The Hidden Treasures and Their Impacts on Sustainable Societies

Zarrabi M

Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: m_zarrabi@royaninstitute.org

Since the era when human being started the formation of the early communities and societies, he was perpetually confronted with threats coming from unknown and mysterious resources and reasons which put human's existence into various hazardous condition and got him baffled as he could find no solution to tackle them. However, throughout the history, as the science and technology got improved, some of these life threatening problems faded, though no completely. But, as a matter of fact, there raised new types of diseases which looked much more complicated than the previous ones in so many features making them so tough to be tracked and treated.

Emerging the industrial era, some sorts of terminal diseases came into existence and it was followed by a raise in death rate among the nations regardless the race, development status or geographical location. Now it was time for the researches and scientists of medical science to take crucial steps in this international arena and take up the cudgels on behalf of human health which is considered as the leading factor in creating a sustainable society. Bringing this fact into account that enjoying a sustainable society leads to a happier population and as its consequence a great help to boost the GDP and GNP of countries, all global scientific bodies reached this consensus to follow up a series of studies and research to cope with these constructive factors.

The point not to be neglected is that God has gifted human being with a vast variety of abilities and facilities in its surrounding; though it may look a bit time taking to discover them. Among these gifts, we come to the supernatural power of stem cells hidden in various sources such as cord blood, dental pulp or human milk and so forth. The potentials hidden in various forms extracted from these cells are so vast and miraculous which could be exploited in treating some forms of diseases previously thought as the killing ones. Nowadays, the huge rate of research and achievements made on the stem cells have led lots of patients to be cured and hence caused the nations and communities to enjoy a joyful and fruitful life. Thanks to such enormous potentials in their hearts, no doubt stem cells should be called as the hidden treasures for sustainable societies.

Oral Presentation

O_{nm}-1: The Impact of Garlic tablet on Pain in Women with Endometriosis

Behboodi Moghadam Z

Department of Reproductive Health, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran
Email: behboodi@tums.ac.ir

Background: Endometriosis is characterized with both proliferation of endometrial glands and stroma outside of pelvic along with inflammatory reactions and the formation of fibrotic tissue. Symptoms associated with the disease include chronic pelvic pain, dysmenorrhea, dyspareunia, pain during bowel movements and infertility. In these days, people would like to use herbal medicines, this study aimed to investigate the effects of garlic tablet on pain of women with endometriosis.
Materials and Methods: This study was a triple blind clinical trial with 120 women of 20-45 years old suffering dysmenorrhea, dyspareunia, and chronic pelvic pain caused by endometriosis visited in Valiasr Reproductive Health Center who were referred in 2015. The samples were randomly divided into intervention and control groups. At first, pain intensity of all members was assessed with a visual analogue scale pain and women with pain scale equal 4 and more were enrolled. All volunteers were received garlic tablets or placebo every day over a 12 week period. The researcher called the women at the end of 4, 8 and 12 weeks and asked them the mean pain scores. Difference between pain scores of both groups was investigated by using the visual pain scale tool over 12 weeks. Data analyzed by SPSS software (version 22) and P-value less than 0.05 was considered as significant.
Results: Before the intervention, both groups were similar in mean scores of pain (dysmenorrhea, dyspareunia, chronic pelvic pain) ($P>0.05$). The results showed that the pain score of intervention group decreased significantly after receiving garlic tablets in the first, second and third measurements of pain (dyspareunia, dysmenorrhea, chronic pelvic pain) ($P<0.05$). Repeated measures ANOVA showed the decrease in pain score is more significant than control group ($P<0.05$).
Conclusion: Garlic can be used as an herbal medicine which is effective on decreasing of pain (dysmenorrhea, dyspareunia, chronic pelvic pain) that are a result of inflammation and an imbalance between oxidant and antioxidant factors such as endometriosis.
Keywords: Endometriosis, Dysmenorrhea, Dyspareunia, Chronic Pelvic Pain, Garlic Tablets

O_{nm}-2: Prognostic Value of Clinical Symptoms in Diagnosis of Endometriosis: A Three-Yyear Study

Javam M, Niknejadi M, Rashidi Z*, Niknejad F

Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: zohrehrashidi70@yahoo.com

Background: This study was performed to investigate various symptoms of endometriosis among infertile women.
Materials and Methods: This was a prospective study conducted at Royan Institute, Iran, from April 2010 to March 2013. All infertile women suspected of having endometriosis, who were booked for laparoscopy, were invited to the investigation. An interview was done with each patient about history of the symptoms to detect clinical manifestations of the disease. Frequency of each symptom was calculated with SPSS18 software. A logistic regression analysis was performed to study the prognostic value of each symptom.
Results: Total numbers of 262 laparoscopies were performed at Royan Institute. Of these, 101 patients met the inclusion criteria, and were recruited in the investigation. Patients aged 30.78 ± 4.79 years in average and the duration of infertility was 4.20 ± 2.91 years among them. Type of infertility was primary in % 84.21 of cases & secondary in %15.79 of those. Reasons of infertility among study patients were as follow: Male factor (22.8%), uterine factor (11.9%), ovulatory factor (20.8%), tubal factor (30.7%) and multifactorial (13.5%). Clinical symptoms of endometriosis among study samples were: secondary dysmenorrhoea (86.13%), dyspareunia (34.65%), pelvic pain (15.84%), abnormal uterine bleeding (10.89%) and dyschezia (5.94%). As derived by logistic regression analysis, a history of severe dysmenorrhoea, followed by pelvic pain, increases the possibility of the presence of endometriosis (Odds ratio of 27 and 19 respectively).
Conclusion: Our study demonstrated that presence of dysmenorrhea and pelvic pain strongly predicts presence of endometriosis and the likelihood of endometriosis is 27 and 19 times more in these women, respectively. Therefore, they can be used as important criteria for gynecologists and midwives.
Keywords: Endometriosis, Infertility, Laparoscopy

O_{nm}-3: Confirmatory Factor Analysis of A Quality of Life Measure in Women with Polycystic Ovary Syndrome

Nasiri Amiri F[1*], Ramezani Tehrani F[2], Simbar M[3], Montazeri A[4], Mohammadpour R[5]

1. Mental Health Research Group, Health Metrics Research Center, Iranian Institute for Health Sciences Research, ACECR, Tehran, Iran
2. Department of Biostatistics, Health School, Mazandaran University of Medical Sciences, Sari, Iran
3. Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Midwifery, Faculty of Medical School, Babol University of Medical Sciences, Babol, Iran
5. Department of Midwifery and Reproductive Health, Faculty of Nursing and Midwifery , Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: nasiri_fa@yahoo.com

Background: The health related quality of life (HRQoL) among women with polycystic ovary syndrome (PCOS) is reduced due to emotional, psycho-social, infertility, marital and hirsutism problems. The study aimed to investigate exploratory and confirmatory factor structure of HRQoL measure in women with PCOS (PCOSQ-50).
Materials and Methods: A cross-sectional study was conducted to assess the construct validity of the PCOSQ-50. The HRQoL in women with PCOS was designed using a qualitative study. Next, 350 women with PCOS were selected using a convenience sampling method. With the consideration of ethical principles, the women were requested to fill out the ques-

tionnaires. The Kaiser-Meyer Olkin test was used to assess the sampling adequacy and the cutoff point of 0.40 was considered the minimum load factor required for maintaining the extracted factor. In addition, the reliability of the instrument was assessed using internal consistency and test–retest methods.
Results: The principal component analysis led to a six-factor solution including 'psychosocial and emotional', 'self-body image', 'fertility', 'sexual function', 'obesity and menstrual disorder' and 'hirsutism' that jointly accounted for 50.83 percent of the observed variance. The Cronbach's alpha coefficient for six factors was 0.87-0.95 and 0.92 for the whole questionnaire. The 43-item model was supported by the confirmatory factory analysis. In comparison to the 50-item model, the 43-item model had an improvement on several incremental fit indices and achieved a more parsimonious model fit.
Conclusion: The 43-item PCOSQ showed appropriate validity and reliability. Its psychometric quality was superior to the original version. However, its predictive efficacy should be examined using longitudinal studies.

O_{nm}-4: Endometriosis and Femininity: A Qualitative Study.

Riazi H[1*], Tehranian N[2], Ziaei S[2], Mohammadi E[3], Hajizadeh E[4]

1. Department of Biostatistics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Midwifery and Reproductive Health, Faculty of Nursing and Midwifery , Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
4. Department of Nursing, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: h.riazi@sbmu.ac.ir

Background: Endometriosis is a chronic and debilitating disease, which affects all aspects of the women's life. The purpose of the present study was to explore the perception and experiences of endometriosis patients about femininity.
Materials and Methods: A qualitative research was conducted to obtain data from 18 purposely selected endometriosis patients referring to a teaching hospital in Tehran, Iran. Data were collected by in-depth interviews and were analyzed using a conventional content analysis.
Results: Seven categories and three main themes emerged from the participants' experiences. The themes were: 1. Feeling gynecologic disorders included three categories: menstrual disturbances, complaint of the irritating cyst and pelvic infection problems, 2. Disruption in marital life included two categories: dyspareunia and infertility, and 3. Disrupted social life included two categories: emotional and communicational disturbances and impairment in daily activities.
Conclusion: The findings of the present study showed that endometriosis affects femininity, which may have devastating consequences on individual, family and social life of affected person.
Keywords: Endometriosis, Femininity Role, Qualitative Research

O_{nm}-5: The Necessity of Establishing A Comprehensive Gamete/ Embryo Donation Information Bank in Iran

Yahyaei A[1, 2*], Kariman N[2], Kiani M[3]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Midwifery and Reproductive Health, School of Nursing & Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Medical Ethics, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: azar.yahyaei@yahoo.com

Background: After almost four decades of using assisted reproductive technology via gamete/ embryo donation and the birth through this method, international community experience indicates that establishing a comprehensive Gamete/ Embryo donation information bank can partially overcome the adverse consequences of assisted reproductive technology and partially respond the needs of donating parties including the donor / recipient and those born by this method. Yet, despite nearly a decade of gamete/ embryo donation application in Iran, although the international community experience is available to deal with the adverse consequences of assisted reproductive technology, no measure has been taken into account at national level by the infertility treatment policy makers.
Materials and Methods: This research is a descriptive study of quality of services provided in 11 embryo donation infertility centers in Oct 2013 – July 2014 by interviews with 100 couples seeking embryo donation in the Tehran central forensic center. The embryo donation instructions of these centers were collected. The research instrument was two questionnaires which assess ethics of embryo donation processes in infertility centers. The findings were analyzed using descriptive statistics indices.
Results: The study shows that most infertility treatment centers suffer from lack of guideline in terms of evaluating the physical and mental health of embryo donors / recipients, ensuring the health of donated embryo, and adapting donor/recipient characteristics. No measure has been taken into account to prevent the marriage of those born via embryo donation with their biologic brothers or sisters and quasi-inheritance. Concerning the financial dimensions of embryo donation process, the study indicates embryo sales by donors. Here, infertility treatment center plays the role of intermediaries. Critically important is the disconnection between infertility treatment centers and embryo donors / recipients after donation.
Conclusion: Comprehensive Gamete/ Embryo Donation Information Bank needs to be established for standardizing the donation process and realistic acceptance by users, and ensuring the interests of those born with this method in Iran by infertility treatment policy makers.
Keywords: Ethical Principles, Embryo Donation, Infertility

Poster Presentation

P_{nm}-1: Vaginal Dryness and Female Sexual Dysfunction in Infertility from Traditional Persian Medicine Point of View

Akhtari E

Traditional Medicine Department, Traditional Medicine Faculty, Iran university of Medical Sciences, Tehran, Iran
Email: akhtari.e@iums.ac.ir

Background: Sexual life and satisfaction is one of the main aspect of fertility. Normal vaginal discharge can make sex more enjoyable than vaginal dryness state. So attention to vaginal natural vaginal discharge in the infertility management period can increase rate of succeed because of couple satisfaction of sexual life.
Materials and Methods: Traditional Persian medicine as a complementary medicine has a natural ways to manage vaginal dryness that its rate is higher than in infertility. So as a library research, it was searched and studied all the reliable and available traditional medicine texts about the natural ways to manage vaginal dryness and all the datas were gathered.
Results: Herbal remedies are a main part of traditional Persian medicine. One of the available herbal remedies to manage vaginal dryness is sitzbath with warm boiled extract of special flowers. These flowers have subtlety, viscosity, fragrance and softness so its property appears in actions and reactions between them.
Conclusion: Traditional Persian medicine can plan a herbal management for vaginal dryness that it is without side effects. Actually the physicians can advise herbal remedy to make more satisfaction in couple's sexual life that it manages with sitzbath of boiled flowers.
Keywords: Traditional Persian Medicine, Vaginal Dryness, Herbal Medicine, Sexual Life, Satisfaction

P_{nm}-2: Management of Genital Tuberculosis in Women with Infertility

Arianfar Z*, Irani Sh, Ahmadi F

Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: z.arianfar2491@gmail.com

Background: The incidence of tuberculosis has been increased in recent years and the World Health Organization has been paid much attention to genital tuberculosis. Tuberculosis is one of the most important infectious diseases of the respiratory system worldwide that may travel through the bloodstream, leading to contamination of the reproductive system and finally Genital tuberculosis leads to infertility. The aim of this study is management of genital tuberculosis in women with infertility.
Materials and Methods: A narrative review was performed within articles published a “PubMed “, “Elsevier”, “SID “and original text books to reach the aim.
Results: The first step in collision with infertile women with suspected genital tuberculosis, is Taking a careful history .The history of infectious diseases and contact with infected people. The next step in the diagnosis of tuberculosis is pelvic exam and diagnostic tests that can be helpful. The most common location of genital tuberculosis is tubal. It is usually asymptomatic, but chronically with symptoms such as Pelvic pain, irregular vaginal bleeding and oligomenorrhea. The first diagnostic test, is tuberculin skin test and look for the positive test, hysterosalpingography(HSG) can be helpful .Genital tuberculosis has a wide range of radiological manifestations with slow growing symptoms and a diagnostic laparoscopy is used.
Conclusion: Imaging methods, especially HSG can be very helpful in the diagnosis of genital tuberculosis but is better, to use along with other diagnostic methods.
Keywords: Genital Tuberculosis, Hysterosalpingography, Clinical Test

P_{nm}-3: One-Child Policy, Reproductive Right, and Development Challenges

Behboodi Moghadam Z, Rezaei E

Department of Reproductive Health, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran
Email: rezai520@yahoo.com

Background: One of the most significant demographic changes of the past 3 decades is the sharp decrease in fertility throughout the world, especially in developing countries. In Iran, in line with these changes, the total fertility rate per woman dropped to 1.8 in 2009 from 7.7 in 1966. Moreover, the World Bank has estimated Iran's population growth rate to have dropped to 1.95% in 2010-2014 and to further drop to 1.23% in 2015-2019 and to 1.13% in 2020-2024. Moreover, the World Bank has estimated Iran's population growth rate to have dropped to 1.95% in 2010-2014 and to further drop to 1.23% in 2015-2019 and to 1.13% in 2020-2024. World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the United Nations Population Fund (UNFPA), can play a critical role by providing factual support, as well as the weight of professional authority. Three basic principles (the key to improving reproductive health is women's autonomy, standing and addressing reproductive health in the way women experience, and connect the different international, country, community levels). can be distilled from what several commentators have called "women-centered" approaches to reproductive health. Each has important implications for the analysis of the connections between law and health.
Materials and Methods: The intent of the study was to review the current level of evidence regarding One-Child Policy, Reproductive Right, and development challenges. Eight databases (Scopus, CINAHL, Medline, ProQuest, EMBASE, PsycINFO, Cochrane, Web of Science and Communication and Mass Media Complete) were searched from 1990 to present using a comprehensive search strategy.
Results: The results of the different studies revealed that marital age, marital satisfaction, social support, economic status, hopefulness and quality of life have a direct influence on the timing of the first birth, with marital age exerting the highest effect. Being religious may be also a source of discordance in the couples’ reproductive plans. pragmatic approaches adopted by the ruling Shiite ulama to solve some of the social and medical challenges of a rapidly modernizing society may be extended to overcome ethical hurdles faced by the reproductive health program. The most reported reasons for another child such as

the sense of being alone, the desire for pregnancy, spouse request, fertility control goal, need to increase the warmth and happiness in the family. Also the couples' intentions to have a child are more exposed to a partners' conflict if the woman works because the working women have the double role of both contributing to the financial situation of the household and of being the main responsible persons in the childcare. At the end of 2015, China put an end to the one-child policy (OCP). It has resulted in the so-called 4-2-1 family structure, in which the only children had four grandparents and two parents to care for them when they grew up, but where they have to shoulder the care of four grandparents and two parents when they come of age, because both cultural values and legal prescriptions foresee reciprocal care responsibilities between generations. This has led to an enormous care deficit, which triggers conflicts between both genders and generations.
Conclusion: Policymakers should adopt strategies that help women bear their desired number of children within an appropriate time frame and that also facilitate the first childbirth decision-making in women choosing to pursue higher education and hold an employment.
Keywords: One-Child Policy, Childbearing , Couple's Reproductive Decisions, Fertility Intentions

P_{nm}-4: A Review of Traditional Medicine in Infertility (A Systematic Review)

Farh Abadi M*, Zarei M, Rezaie M, Rezaie A

Department of Midwifery, Azad University of Isfahan, Khorasgan Branch, Isfahan, Iran
Email: mehnoosh.farahabady@yahoo.com

Background: Considerable prevalence of the infertility in Iran and worldwide, with respect to the importance of the individual and social problems and economic burden resulting from ARTs motivated the couples to find the treatments with fewer side effects, lower costs, more efficient, and with less difficulty. Thus, traditional medicine has become more important. This study investigates the effect of traditional medicine on the infertility.
Materials and Methods: This review was conducted using Google Scholar, SID, PubMed, and Magiran and searching keywords including infertility, traditional medicine, medicinal plants and temper and 112 papers were extracted. There was no limitation regarding the years when the study was carried out. Then, based on inclusion criteria and exclusion, 60 papers were excluded. After studying the remaining papers by two authors and based on the checklist of the reviews and investigating the different viewpoints by the third author, 38 papers were selected.
Results: Findings: infertility occurs both in men and women resulting from the sperm and ovule and their role in infertility has been emphasized. It also depends on the health of the main organs of the body (heart, brain and liver). Balancing the temper of the organs or using the appropriate nutrients are prioritized in treatment. Different treatment techniques using the plants including saffron, black seeds, fennel, tribulus terrestris, evening primrose, licorice and so on and the manual methods such as bloodletting, cupping and massage.
Conclusion: This study investigates the causes of infertility and it is an introduction to the recognition of the treatment methods from the viewpoint of the traditional medicine of Iran that is a kind of the holistic traditional schools. Due to the expertise of the physicians of traditional medicine and using the medicinal plants with fertility feature, they can be used for future researches or as the alternative or complementary choice for chemical drugs.
Keywords: Infertility, Traditional Medicine, Medicinal Plants, Temper

P_{nm}-5: Consequences of Infertility in Different Cultures

Foroutani MR*, Poorkiani M, Shoyokhi M

Department of Nursing, Larestan Nursing College, Larestan School of Medical Sciences, Larestan, IRAN
Email: larres@sums.ac.ir

Background: Infertility has had different consequences in different cultures. However, all they have in common is trying to get rid of this interpretation and notoriety. Each of the cultures presents some recommendations that are mostly about women and pay more attention to psychological, and sometimes, superstition aspects of infertility.
Materials and Methods: For example, in some parts of southern Iran, people believed, if an infertile woman would accompany women who had a baby to the bathroom would effect on the infertility treatment.
Results: Using herbal medicines such as Teucrium Polium along with other Pharmaceutical Formulations has also been recommended. However, local, none healthy, and in some cases, threatening recommendations have been endangering health of women. These treatments though effective in some cases, so far, there has been no scientific evidence which confirm the effectiveness of the treatments.
Conclusion: Todays, regarding the development of medical treatments, local treatments do not receive attention as before. However, modern treatment multiple failures tempts couples to prefer traditional treatments. Managing and consulting the couples and clarifying the effectiveness and success of the scientific infertility treatments might prevent such pharmaceutical abuse and unpleasant consequences.
Keywords: Infertility, Consequences, Cultures

P_{nm}-6: Melatonin Effect on Oxidative Stress in Infertility Treatment

Janati Ataei P

Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: padidehjanati@yahoo.com

Background: In recent years, infertility treatment has become more acceptable and with improvements in technology, the success rates have been increased. More recently, it has been discovered that an imbalance of reactive oxygen species, or 'oxidative stress', can have a negative impact on the success of infertility treatments, and furthermore, investigators have begun addressing potential mechanisms of preventing these effects with the use of novel oxygen scavengers such as melatonin.
Materials and Methods: This study reviewed the latest published literatures that investigated the effect of melatonin on oxidative stress in infertility treatments.
Results: Melatonin, a pineal hormone that regulates circadian rhythms, has also been shown to play an essential role in the pathogenesis of many reproductive processes. High-concentration melatonin exists in human preovulatory follicular fluid

and melatonin receptors are present in ovarian granulosa cells, which indicates the direct effects of melatonin on ovarian function. Oxidative stress occurs at many levels during the treatment, of infertility. Interventional studies have begun recently, with an emphasis on oral supplementation of melatonin during the ovarian stimulation phase of the IVF cycle and its effects on gamete and embryo quality. Melatonin and its metabolites, as powerful antioxidants and free radical scavengers, can potentially inhibit premature ovarian failure and in combination with progesterone, it has the ability to suppress ovulation in humans, possibly by interfering with LH release.Clinical studies also suggest that melatonin supplementation in IVF may lead to better pregnancy rates. It is well known that shift-workers are more likely than daytime workers to experience circadian disruption and longer menstrual cycles, more menorrhagia and dysmenorrhoea. Suggesting that the menstrual irregularity associated with shiftwork could be explained by melatonin fluctuations. Melatonin, through its neutralization of reactive oxygen and nitrogen species, has been shown in both animal and human studies to improve seminal quality *in vitro*.
Conclusion: Melatonin shows as an adjunctive therapy in the treatment of infertility. Its unique anti-oxidative characteristics and safety profile make it an ideal adjuvant therapy to be further investigated in well designed randomised controlled trials.
Keywords: Melatonin, Oxidative Stress, Infertility, Treatment

P_{nm}-7: The Role of Vitamin D in IVF Outcomes

Janati Ataei P

Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: padidehjanati@yahoo.com

Background: It is estimated that 15 % of couples suffering infertility and infertile women have low vitamin D level. In recent years, *in vitro* fertilization (IVF) has become a main treatment for infertility. It has recently been proposed that vitamin D may be a potential adjunctive treatment to improve IVF outcomes in humans.
Materials and Methods: A review of the literature was conducted by using Embase, Pubmed, and Cochrane database for relevant English language publications.
Results: The relationship between vitamin D and IVF outcomes (clinical pregnancy and live birth) has been investigated, but the association revealed inconsistent. Some studies showed positive correlation between the level of vitamin D in serum and follicular fluid and tendency to achieve clinical pregnancy following IVF. Whereas anther studies found that vitamin D deficiency did not play an important role in the outcome of ART and there was a negative effect of vitamin D on the quality of embryos. Vitamin D deficiency is frequently observed during the reproductive period, with 20–90% of reproductive-age women in North America being deficient. Nowadays, the optimal concentration of 25(OH)D during the reproductive age is still debated. The Endocrine Society suggests a daily intake of 1500–2000 IU of vitamin D3 for women aged 18 to 70 years to reach the optimal 25(OH)D concentration of 30 ng/ml. The same recommendations were suggest for pregnant and lactating women.
Conclusion: In conclusion, infertile women with serum vitamin D deficiency was not correlated with lower clinical pregnancy rates, but related with live birth rate, and serum vitamin D levels higher than 20 ng/ml have been shown to be beneficial live birth rate. Large-scale randomized trials are therefore the next step in determining the optimal levels of vitamin D and the possible adverse effects of vitamin D deficiency. If evidence shows beneficial effects, vitamin D supplementation could offer a practical, simple, and cost-effective means of improving infertility procedures outcomes.
Keywords: *In Vitro* Fertilization, Vitamin D, Outcome

P_{nm}-8: Psychological Effects of Male Infertility

Jannesari Sh

Department of Midwifery, Faculty of Nursing and Midwifery, Shahid Beheshti University, Tehran, Iran
Email: shararehjannesari@gmail.com

Background: When a man finds out he is infertile, it often is completely unexpected and comes as a complete shock, especially since it is still incorrectly believed that fertility issues are due to female conditions. Men undergo various battles when facing personal infertility. These battles include anxiety concerning potency, masculinity, and sexual adequacy
Materials and Methods: This study was conducted by library source and paper indexed in medline to describe Psychological effects of male infertility
Results: Feelings of stress, depression, guilt, or anxiety in infertile men can cause psychogenic impotence, which heightens the feelings of inadequacy that already accompany infertility. The psychological stress of infertility has been shown to affect sperm parameters in significant and demonstrable ways that may further contribute to difficulties with erectile potency. The diagnosis of infertility causes many males to question their masculinity. Male factor infertility is frequently associated with high levels of stigma. Many people assume that infertile men cannot perform sexually. This stigma adds to the heightened insecurities in infertile men. However, infertile men are likely to be depressive and anxious, and have lower masculinity scores and self-esteem. Couples with long term infertility, who have faced much treatment failure, report higher levels of depression, low satisfaction with their sex lives, and low levels of well being.
Conclusion: A lot of men believe that there are numerous disincentives to psychological treatment despite its potential benefits, especially for those forms of infertility most linked to psychological and behavioral factors. Men who acknowledge infertility, articulate the sources of their anxiety, express their loss of confidence in sexual adequacy, deal openly with their wives' disappointment and anger, and consciously redefine their male and marital roles show improved sperm counts and may even be more successful at impregnating their wives. There is an important role of psychoanalytic treatment when dealing with male infertility.
Keywords: Infertility, Male, Psychological

P_{nm}-9: The Benefits of Psychological Counselling during Fertility Treatment Introduction

Khoramabadi KH

Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: ms.khoramabadi@yahoo.com

Background: The clinical definition of infertility as used by the World Health Organization (WHO) is "a disease of the reproductive system defined by a failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse". The absolute number of couples affected by infertility increased from 42 million in 1990 to 48/5 million in 2010. Embryology estimated that up to 2011, approximately 5 million children had been born using ART. IT is observed that couples undergoing infertility treatment suffer various physical and psychological difficulties at a higher frequency on both men and women than the comparable general population. There are many reports on psychological mood among infertile patients, but research that examines couples' psychological experiences during the infertility treatment process is limited. Thus we study about these problems.
Materials and Methods: This review article has been extracted from 27 article that has indexed in most valid scientific cites that has published from year 2014 to 2017.
Results: Millions of couples, who get married with the hope to plan a family of their own, come to realize that they are infertile. Women who have participated in an *in vitro* fertilization (IVF) program indicate that the treatment is one of the most stressful experiences of their lives. The success or failure of infertility treatment is an important factor that can exacerbate the psychological distress of individuals and couples. Chances of conceiving are around 72% if infertile couples are willing to undergo repeated fertility treatment cycles. However, the proportion of patients who fail to comply with recommended treatment is around 15% for intrauterine insemination (IUI) and 22% for assisted reproductive treatment (ART). Infertile couples often experience emotional and physical distress. The most common reactions to emotional distress related to infertility were shock, anger, guilt, lowered self-esteem, sexual dysfunction, marital dissatisfaction and social isolation. Further, the complexity and high cost of treatment and social pressure to have children can be additional stressor on the marital relationship and quality of life. The association between psychological factors such stress and depression and menstrual disturbances response to ovarian stimulation has been noted. Yet, the direction of this relationship is unclear as infertility may lead to secondary depression and significant stress. these factors with lack of knowledge regarding them to stop treatment. Thus infertility programs should offer and encourage patients to participate in professional counseling services for preventive and remedial purposes. therapeutic counseling may be more effective if initiated before the infertility treatment. Women's present levels of distress and coping strategies should be assessed prior to initiating infertility treatment to provide the patients with opportunities to learn and practice new adaptive behaviors that could enhance their ability to cope with infertility and the associated medical procedure. The early identification of patients at risk for psychological maladjustment is important because it enables fertility staff to offer additional care to these patients in order to prevent such problems and ease their experience of treatment. Acupuncture may be a useful intervention to facilitate the reduction of infertility related stress. it is evident that the inclusion of counseling in infertility programs could benefit all participants involved and result in increased satisfaction with infertility treatment procedures.
Conclusion: Infertile couples must refer to the psychological burden of treatment as one of the most important reasons for withdrawal from recommended treatment. The SCREENIVF can be used before treatment to screen patients at risk for psychological maladjustment by assessing five risk factors: anxiety, depression, helplessness and lack of acceptance cognitions and social support. Psychological support must be done to all couples from the early stages of the diagnostic and therapeutic process of infertility. Such an approach could decrease the number of couples who refuse a subsequent treatment cycle owing to the psychological burden of previous treatment failure. In conclusion, must be ensuring that psychological support can become an integral part of infertility treatment.
Keywords: Psychological Counseling, Fertility, Treatment

P_{nm}-10: The Impact of Advanced Paternal Age on Infertility

khoramabadi M

School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Shahid Beheshti University, Tehran, Iran
Email: ms.khoramabadi@yahoo.com

Background: Some data point towards a worldwide trend of conceiving children later on in life. The recent trend toward delayed parenthood raises major concerns because of the adverse effects of aging on couple fertility. Therefore, attention should also be paid to the influence of advanced paternal age on reproductive outcome. It is demonstrated that aging clearly affects female fertility, but can also affect male fertility. A few studies have concluded that paternal age affects the risks of infertility and of adverse pregnancy outcome. Thus, it is important to identify the risk factors to be faced by couples in which the man is older than 40 years.
Materials and Methods: This review article has been extracted from 27 article that has indexed in most valid scientific cites that has published from year 2012 to 2017.
Results: In industrialized countries, the proportion of couples who delay pregnancy has increased greatly in recent decades. The rise in life expectancy, women's entry into the labor market and the popular use of contraception, has contributed to the social phenomena of delaying family planning and parenthood to the mid or late thirties. Assisted reproductive technologies enabling older couples to hope that they can materialize their aspirations for healthy offspring later in life. The trend of older parenthood is true also for males. Men who expect their first baby at an advanced or very advanced age constitute a socioeconomically heterogeneous group with more health problems and more risky health behaviour than younger men. Since older men often have their first child with a woman of advanced age, their combined risk of adverse perinatal outcomes needs further attention thus the risk of adverse obstetric and perinatal outcomes would increase. Increasing paternal age can be associated with decreasing androgen levels, decreased sexual activity, alterations of testicular morphology and a deterioration of semen quality (volume, motility, morphology). Age-impact on testes and semen parameters may be due to the specific effects of age alone, but can also be based on factors associated with age, as for example vascular diseases, obesity, infections of the accessory reproductive glands or an accumulation of toxic substances. Although several theories have been proposed, the exact mechanisms responsible for the observed age-related decline in male fertility remain to be elucidated. Very few fertility treatments have been done for older infertile men. varicocele repair, lifestyle modifications and antioxidant supplements may improve fertility potential in the aging male. in view of the growing number of older patients attending fertility and assisted reproductive facilities, proper counseling about the implications of age effects on gamete quality should be provided to

both partners prior to family planning and infertility treatment. While efforts towards reducing teenage pregnancy rates is done, little efforts is given on the risks of delaying childbearing until advanced maternal age, and less still on the risks of advanced paternal age. Possible interventions might include health promotion advising people about the risk of delaying childbearing and encourage couples to have children earlier rather than later.
Conclusion: Like maternal age of ≥35 years, paternal age of ≥40 years should be considered to be a key risk factor for infertility. We therefore advocate that young women and men should be given more information about fecundity and the medical risks associated with postponing childbirth. Furthermore, must be sensitive about effective incentives, such as facilitation of parental leave and improved financial conditions, for couples to conceive a few years earlier than is usual in modern societies for childbearing.
Keywords: Paternal Age, Infertility, Impact

P_{nm}-11: Is Trichomonas Vaginalis Cause of Infertility? A Systematic Review

Nourollahpour Shiadeh M[1, 2*], Bagheri M[1], Danesh M[2], Rostami A[3]

1. Department of Reproductive Health, School of Nursing and Midwifery, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Midwifery and Reproductive Health, Nursing and Midwifery School, Mazandaran University of Medical Sciences, Sari, Iran
3. Infectious Diseases and Tropical Medicine Research Center, Babol University of Medical Sciences, Babol, Iran
Email: malihe.nurollahpur@gmail.com

Background: Trichomonas vaginalis is a flagellated protozoan parasite with a worldwide distribution. This organism inhabits the vagina in the female and the urethra, epididymis, and prostate gland in the male. Some studies have reported that tubal infertility is twice as high in women who reported a history of trichomoniasis compared with women with no such infection.
Materials and Methods: A search in the PubMed database and Google scholar was performed on relevant studies published from 2000 to 2016. Articles that were written in English and relevant to the topic were enrolled in this study.
Results: Among women, trichomoniasis may plays an important role in premature rupture of the placental membranes, premature labor, and low-birth-weight infants in pregnant women. It is also associated with cervical neoplasia and atypical pelvic inflammatory disease that these complications can lead to female infertility. This parasite can decreases C3 and C4 (the complement elements) and increases the IgA level in vaginal discharge and serum prolactin. Among men, trichomoniasis has emerged as a cause of non-gonoccocal urethritis and as contributing to male factor infertility. T. vaginalis was found in 28.8 % of male patients with urethral discharge and 8.2 % of these patients suffering from impotence and infertility. This organism increases the seminal fluid viscosity, semen agglutination and percentage of particulate debris that can lead to a decrease in sperm quality and motility. Moreover, it is cause of the changes in normal morphology of sperms (abnormal sperms), viability and membrane integrity. Nevertheless, such complications may be resolved after treatment with one dose of metronidazole. T. vaginalis is able to phagocytose of sperm cells and its byproducts rapidly killed sperms *in vitro* that these effects in humans may contribute to the infertility in infected couples.
Conclusion: The current review indicates that T. vaginalis may be a neglected cause of infertility. Given the widespread prevalence of this infection, we suggest further studies to better understanding of relationship between such infection and infertility.
Keywords: Trichomonas Vaginalis, Cervical Neoplasia, Abnormal Sperms, Infertility

P_{nm}-12: Effects of Acupuncture and Moxibustion on Infertility: Narrative

Soltani M[*]

Bentolhoda Hospital, North Khorasan University, Bojnourd, Iran
Email: Soltanim76@gmail.com

Background: According to the world health organization, the prevalence of infertility in the world is 12 to 15 percent. Infertility affect the different aspects of personality and psychological, functions of family, work and communication. Acupuncture and moxibustion on treatment of infertility has a long history, and researches have progressed a lot on integration treatment of acupuncture and moxibustion and modern gynecology. This study aimed to review the studies on the effect of acupuncture and moxibustion on infertility.
Materials and Methods: I identified relevant studies from databases including the sid, googlescholar, magiran, sciencedirect, pubmed. numerous articles and reviews related studies were selected and finally the results were reported.
Results: 20 clinical trial was conducted and the most points are used on cv(conception vessel), st(stomach), sp(spleen), li(large intestinal), lr(liver) channels. most studies suggest positive effects of acupuncture or moxibustion on the different stages of assisted reproductive technology as well as stress and anxiety in infertile person. but at present, there is insufficient evidence to support the use of acupuncture for treatment of ovulation disorders in women with pcos(polycystic ovarian syndrome).
Conclusion: TCM (traditional chinese medicine) contends that infertility is closely related to liver, spleen, and kidney. Several studies have been conducted with a high reputation in the field of acupuncture and moxibustion effect, and with reference to the fact that in modern obstetrics and gynecology, non-invasive treatment methods and minimal side effects is considered, this method can be considered in treatment as complementary and effective non-pharmacological methods with low side effects, and is expected to be the assistant therapeutic approach for the improvement of infertility outcome. in sum, it seems to be a useful and safe tool and can be an effective option for treating infertility in modern gynecology, while more study needs.
Keywords: Infertility, Acupuncture, Moxibustion

P_{nm}-13: A Review on The Effect of Medicinal Plants on Boosting Male Fertility

Zarei M[1*], Farahabadi M[1], Rezaie M[1], Rezaei A[2]

1. Department of Midwifery, Azad University of Isfahan, Khorasgan Branch, Isfahan, Iran
2. Department of Midwifery Counseling, Azad University of Isfahan, Khorasgan Branch, Isfahan, Iran
Email: zmonire74@yahoo.com

Background: Infertility or reduced fertility is one of the major problems in medical sciences. Male infertility is the main cause

of 30-50% of infertility, mainly due to the impairment in the production of a sufficient count of healthy and active sperms. In recent years, the growing use of medicinal plants has increased the number of the studies on medicinal plants. Therefore, this study aims to review the effect of these plants on boosting the male fertility.

Materials and Methods: This review was carried out by searching the keywords such as male fertility, infertility, medicinal plants, and spermatogenesis in international databases including Science Direct, Pubmed, SID, Medline and Google Scholar; 10 papers were obtained, and after evaluating the inclusion and exclusion criteria, 30 papers were removed and finally, 70 papers were listed from 2005-2016 and data were extracted.

Results: Medicinal plants are compatible with the body because of the natural essence and medicinal homologue compounds together and they usually have no side effects. Medicinal plants boost male fertility and include parsley leaves, garlic, saffron, fumitory, carrot seeds, eucalyptus, sage, pumpkin, ginger, cinnamon and aloe vera. These plants are effective on the spermatogenesis and sexual behaviors by affecting the different parts of the reproductive system.

Conclusion: The use of medicinal plants that boost male fertility can be regarded as an alternative to chemical drugs.

Keywords: Male Fertility, Infertility, Medicinal Plants, Spermatogenesis

Authors Index

COOPERATORS & SPONSORS

Iranian Academic Center for Education, Culture and Research (ACECR)

Vice Presidency of Science and Technology
National Council for Stem Cell Research & Technology

Iran National Science Foundation

Council for Stem Cell Sciences and Technologies

Islamic Development Bank

Iran Science Elites Federation

The Office of President
Vice-Presidency of Science & Technology Iran Natioal Science Foundation

Center for International Scientific Studies and Collaboration
Ministry of Science, Research and Technology

ROYAN STEM CELL TECHNOLOGY CO.

ROYAN
CORD BLOOD
BANK

There is hidden another new life,

in each birth

Do you know?!

Transplantations, applying umbilical cord blood stem cells, play a leading role in curing the patients suffering from various form of blood diseases such as: Blood Cancers, Lymphoma, Thalassemia and so forth.

www.rsct.ir

No: 24,Corner of East Hafez Alley, Banihashem Sq, Resalat High way,
Tehran, Iran Tel : (+98-21),27635000 Fax : 89781308

Royan_cbb

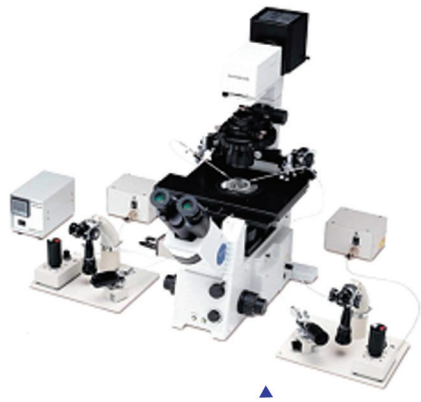
OLYMPUS

Your Vision, Our Future

استریو میکروسکوپ مدل SZX16

میکروسکوپ تحقیقاتی مدل BX63

میکروسکوپ تحقیقاتی مدل CKX53



میکروسکوپ IVF به همراه ملحقات

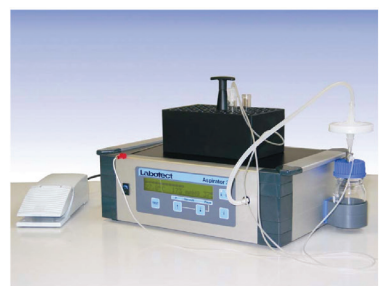
انواع میکروسکوپ‌های تحقیقاتی با کاربردهای مختلف

Labotect

Labor-Technik-Göttingen

انکوباتور CO2 مدل C200

کاتتر انتقال جنین



آسپیراتور

فنون آزمایشگاهی LAB TECH

نشانی: تهران، خیابان سهروردی شمالی، خیابان هویزه شرقی، پلاک ۳۱
کدپستی: ۱۵۵۸۶۱۸۷۹۱ تلفن: ۸۸۷۴۸۰۰۰ نمابر: ۸۸۷۴۸۰۱۰

Diphereline™

triptorelin

- The best choice for treatment of **ENDOMETRIOSIS** with genital and extra-genital localization (from stage *I* to stage *IV*).
- Pre-operative treatment of **UTERINE FIBROMYOMA.**
- Suppression of **OVARIAN FUNCTION.**

IPSEN
Innovation for patient care

DARYAN SALAMAT PHARMA GROUP
#21, No79, Vahid Dastgerdi St., Shariati St.
Postal Code: 1911817616 Tehran - IRAN
Tel: +98 21 22921890-2 Fax: +98 21 22921893

Mail: info@daryansalamat.com

biosafe
Part of GE Healthcare Life Sciences

GE Healthcare
Life Sciences

World Leaders in Cell Processing

PROCESS AUTOMATION WITH SEPAX 2

- FULLY AUTOMATED GMP COMPLIANCE
- CLOSED SYSTEM
- HIGH EFFICIENT CONSISTENT CELL RECOVERIES
- FULLY MOBILE
- FULL TRACEABILITY

تکنولوژی سیپکس جهت فرآوری اتوماتیک

سلولهای بنیادی از خون بند ناف و مغز استخوان

SWISS MADE

PROCESS AUTOMATION WITH SEFIA™

- FAST PROCESSING OF LARGE VOLUMES
- INTUITIVE USER GUIDANCE
- SMART PROCESS CONTROL
- COMBINABLE PROTOCOLS
- TEMPERATURE REGULATION

تکنولوژی سفیا راه حلی تطبیقی جهت

آماده سازی ، جداسازی ، شستشو ، تغلیظ و فرمولاسیون نهائی

هرنوع جریان سلولی در حجم بالا

نمایندگی انحصاری :

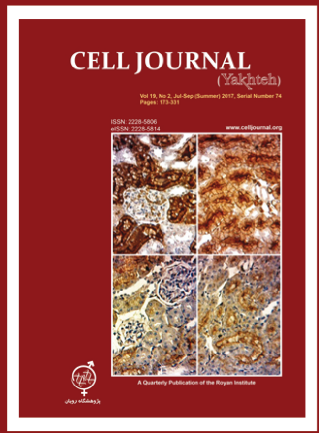
Sinarvin Tajhiz
سینارین تجهیز

تهران – بزرگراه صدر – خیابان قلندری شمالی – کوچه چهاردهم
شماره ۲ – واحد ۷ تلفن : ۲۶۷۶۸۳۲۸–۰۲۱
مشهد – شهرک فناوری بیوتکنولوژی شمال شرق – واحد ۳۱۳
تلفن : ۳۸۶۹۶۸۹۶–۰۵۱ و ۳۵۴۲۴۴۷۶–۰۵۱ فاکس : ۳۵۴۲۴۴۷۶–۰۵۱

www.biosafe.ch
www.sinarvin.ir

Journals of ROYAN INSTITUTE

Prize for the Best Article

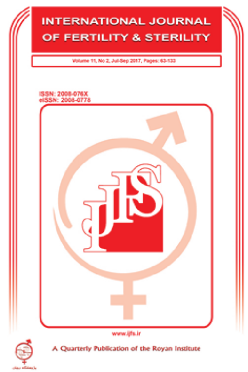


CELL JOURNAL (Yakhteh)

Cell J is a quarterly English publication of Royan Institute in the field of the Cellular and Molecular Science. This journal is a member of the Committee on Publication Ethics (COPE) and indexed in:

1. Thomson Reuters **(ISI)**–> **Impact Factor : 1.636**
2. PubMed and PubMed Central (PMC)
3. National Library of Medicine (NLM)
4. Biosis Preview
5. Index Medicus for the Eastern Mediterranean Region(IMEMR)
6. Index Copernicus International
7. InfoBase Index
8. Cambridge Scientific Abstracts (CSA)
9. EMBASE
10. Scopus
11. CINAHL Database
12. Google Scholar
13. Chemical Abstracts Service
14. Proquest
15. Directory of Open Access Journals (DOAJ)
16. Open Academic Journals Index (OAJI)
17. Directory of Research Journals Indexing (DRJI)
18. Scientific Information Database (SID)
19. Barakatkns
20. Regional Information Center for Sciences and Technology (RICeST)
21. Islamic World Science Citation Center (ISC)
22. Magiran
23. Science Libarary Index

www.celljournal.org



INTERNATIONAL JOURNAL OF FERTILITY AND STERILITY

Int J Fertil Steril is a quarterly English publication of Royan Institute in the field of the Fertility and Sterility. This journal is a member of the Committee on Publication Ethics (COPE) and indexed in:

1. Emerging Sources Citation Index **(ESCI, ISI)**
2. PubMed Central (PMC)
3. National Library of Medicine (NLM)
4. Index Medicus for the Eastern Mediterranean Region(IMEMR)
5. Index Copernicus International
6. InfoBase Index
7. EMBASE
8. Scopus
9. CINAHL Database
10. Google Scholar
11. Proquest
12. Directory of Open Access Journals (DOAJ)
13. Open Academic Journals Index (OAJI)
14. Directory of Research Journals Indexing (DRJI)
15. Scientific Information Database (SID)
16. Barakatkns
17. Regional Information Center for Sciences and Technology (RICeST)
18. Islamic World Science Citation Center (ISC)
19. Magiran
20. Science Libarary Index

www.ijfs.ir

TAIMAZ

ZEISS

لحظــه ای که دقت و کیفیت با تکنولــوژی پزشکــی ادغام می گــردد،
لحظــه ای است که ما برای آن می کوشیــم.

Axio Observer
Microscopy

IVF, ICSI, IMSI
flexibility: well-considered
modularity & complete
integration

LSM 880
Microscopy

Your new standard for
fast & gentle confocal
imaging

Axio Vert.A1
Microscopy

Your inverted microscope
for advanced routine

شرکت تایمــاز، نماینــده انحصــاری کمپانی زایــس آلمــان

www.taimaz.com | Tel: 021-88382820-38

FERRING
REPRODUCTIVE HEALTH

Decapeptyl 0.1®*
triptorelin

For a successful
Pituitary down-regulation...

01 Accurate measured dose.
(No dose adjustment is needed).

02 Simple dose administration.
(Ready to use, no need for preparation).

03 Minimum dose loss and any risk of contamination.
(Ready to use, no need for preparation).

04 Patients' convenience.
(Once daily administration).

FERRING
PHARMACEUTICALS