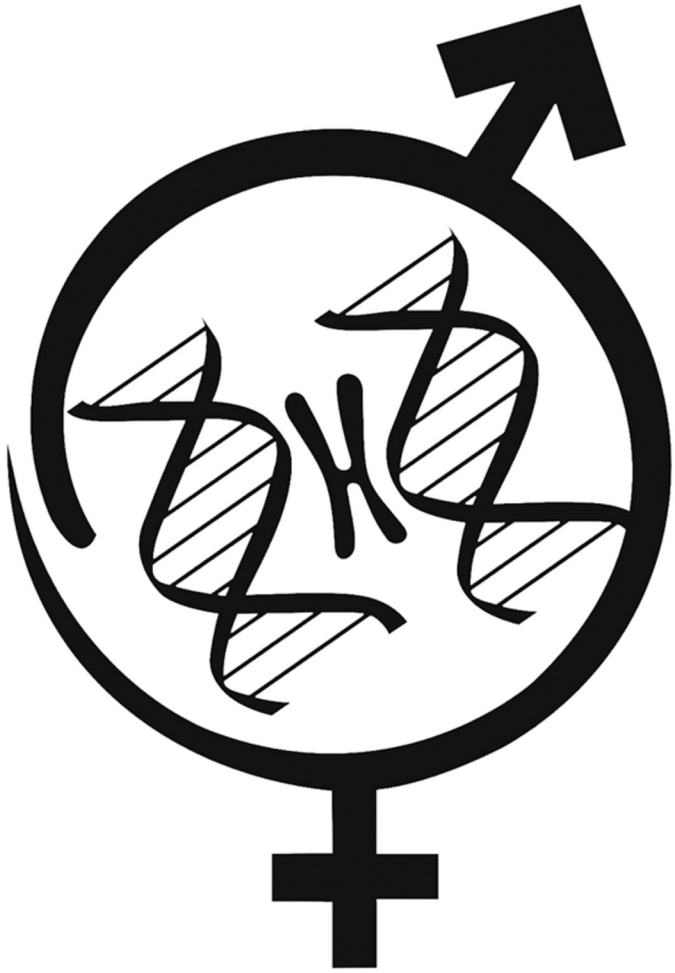
Abstracts of

Royan International Hybrid Twin Congress

24th Hybrid Congress on Reproductive Biomedicine
31 August-1 September 2023

18th Seminar on Nursing and Midwifery
31 August 2023



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Abstracts of the
24th Hybrid Congress on Reproductive Biomedicine
31 Augugst-1 September 2023

18th Seminar on Nursing and Midwifery
31 August 2023

Contents

Scientific Board

Congress Chairperson

Mohammadreza Zamanian

On behalf of the Organizing Committee, it is my pleasure to invite you to **24th Royan International Congress on Reproductive Biomedicine (31 August- 1 September 2023), Tehran, Iran**.

In **2022**, we were delighted to welcome more than 1500 participants who joined our 23rd congress from more than 17 different countries. This was our first hybrid congress which was held following two consecutive virtual ones due to COVID 19 pandemic, and we hope this new experience has been stimulating and enriching for the participants.

Once again, in 2023, the Royan International Congress will be officially held in a "hybrid format" which combines "in-person" with "online" sessions. However, we will be looking forward to hold the event, this year as in-person as possible to be able to prepare for meeting up with the pioneers and well known researchers of the field, **face to face**, to discuss the latest scientific updates in **reproductive health**.

The scientific committee seeks out international experts to hold a comprehensive and useful program, including the male and female infertility, clinical embryology and reproductive genetics. The program consists of state-of-the-art lectures, debates, and oral/poster presentations on issues of interest from the infertility field to facilitate the use of novel methods to better understand the basic underpinnings of the ART and ascertain the best practices for clinical management.

The 24th Royan Congress (31 August-1 September 2023) will be guided by the motto **"Let Our Hopes Shape the Future"**.

We are eager to meet you whether in person or on-line soon in the city of thoughts; **Tehran!**

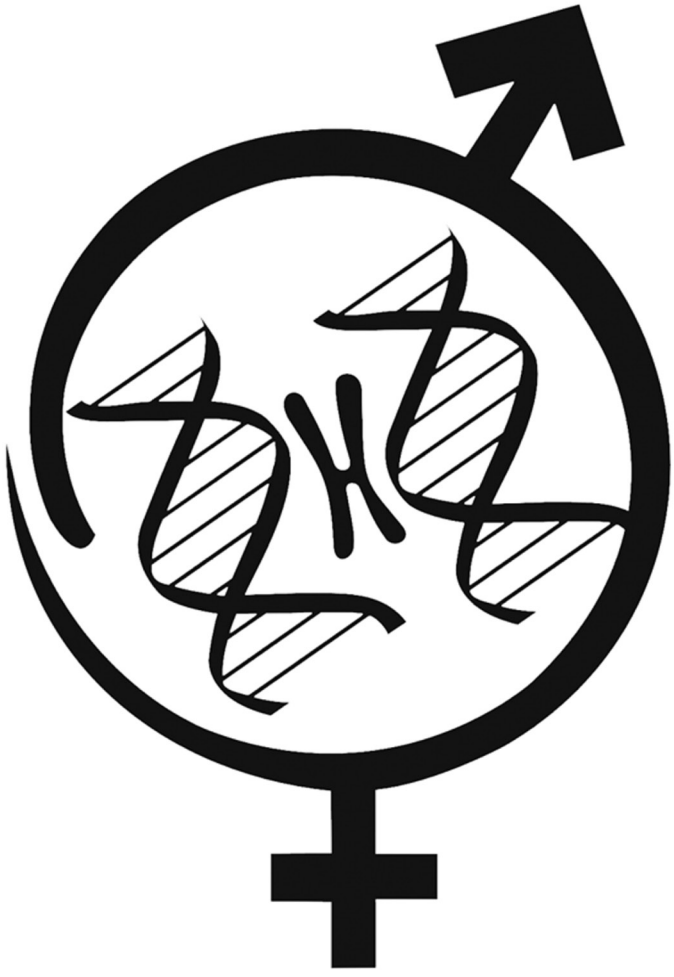
Best regards,
Mohammadreza Zamanian, MD, PhD
Chairperson of the
24th ROYAN International Congress on Reproductive Biomedicine (2023)

Abstracts of

Royan International Hybrid Twin Congress

24rd Hybrid Congress on Reproductive Biomedicine

31 August - 1 September 2023



Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speakers

Andrology

I-1: Personalized Medicine in The Treatment of Infertility: The Use of Artificial Intelligence in The Field of Andrology

Garrido Puchalt N

Director of IVI Foundation, Director of Research Administration at IVI RMA Global, FIVI Valencia Member of the Scientific Advisory Board, Valencia, Spain
Email: Nicolas.Garrido@ivirma.com

Male infertility has been steadily increasing the past few decades, becoming a public health concern.
Few cases have a clearly identifiable cause, while many of them are yet cataloged as idiopathic, and it is assumed that male infertility can depend on several different factors and processes, assumed to be multifactorial, and there may be a number of reasons leading to the male infertile phenotype The basic sperm analysis has limited predictive value over the achievement of success in either natural or assisted conception, in part because several non-measured molecular factors within the sperm cells are crucial to succeed, and identifying the most adequate spermatozoa to fertilize an egg and guarantee success is also a key step on the assisted reproduction process.
To improve the treatment of male infertility and performance of ART, there are several aspects susceptible of being improved.
For instance, we need to ascertain what does a specific sperm cell need to succeed, and then find ways to select the best among millions, finding them within the testicular tissue if they are just a few, or even knowing how to create spermatozoa if they are not produced at all. And this may be needing an individual and personalized approach in each case.
So far, several sperm phenotypical and molecular processes involved on spermatogenesis and correct sperm function and markers of fertility have been described as being related with reproductive success: DNA polymorphisms, DNA integrity, proteomic and metabolomic profiles, membrane charge, apoptotic traits, hyaluronic acid receptors, platelet activating factor, and a long etc.., and some can also be used to select sperm individually for using them in assisted reproduction techniques. Nevertheless, robust evidence for significant improvement in reproductive performance after its application is scarce, or almost inexistent.
In this complex context, new methods providing massive biological data such as genomic sequencing, image analysis and time lapse, and mass spectrometry have driven dramatic increases in the amount of information available to scientists and health care professionals potentially permitting more refined diagnoses and increased therapeutic precision.
Analytic tools have been improved in parallel to match the size, speed, and variety of these molecular and image “big data.”
Among them, machine learning has proved especially valuable, and computer systems use large amounts of data to build predictive statistical models that are iteratively improved when new data are added.

I-2: Sperm DNA Fragmentation: Clinical Aspects and Diagnosis

Sajjadi H

Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: hesam1979@yahoo.com

Infertility is a global health problem, defined as the inability to get pregnant after one year of unprotected sexual intercourse. About fifteen percent of couples suffer from infertility worldwide and male factors account for fifty percent of the cases.
DNA fragmentation index (DFI) reflects the integrity of the DNA, the genetic material of the sperm and its crucial indicator in evaluating semen quality. The sperm DFI is used to assess the DNA damage and directly reflects the degree of sperm DNA destruction.
Semen analysis alone can only provide limited information for the assessment of male fertility, and it does not fully reflect the fertilization potential of the sperm.We need better clinical indicators to determine the cause of male infertility and its relationships with reproductive outcomes. DNA fragmentation may be associated with increased risk of miscarriage (Simon et al) but its association with RIF has not been established. Some studies demonstrated that high DFI is correlated with impaired preimplantation development (fertilization rate), a low rate of good-quality embryos and poor implantation,misscariage rate(pregnancy loss) and low live birth rate. (Niu et al; Velez de la Calle et al; Zini, Boman, Belzile, & Ciampi,). However, some studies have shown that DFI is not associated with a high risk of pregnancy loss. Overall ,we can use DFI as an adjunctive to semen analysis in men with male infertility but in some special cases and limited indications. but the role of DFI on pregnancy outcomes is controversial and requires further investigation.

I-3: Sperm DNA Fragmentation: Impacts of Life Style and Effects on Next Generation

Nasr Esfahani MH

Department of Reproductive Biotechnology, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Many years have elapsed since the initial implementation of the first test aimed at evaluating oxidative stress and chromatin damage in sperm. However, its practical implications within clinical settings have largely remained a subject of inquiry in the background literature. This can be attributed primarily to the intricate and multifaceted aspects of reproductive processes. Recent investigations have illuminated the significance of factoring in DNA fragmentation in sperm alongside variables like the ages of both male and female partners. When accounting for these confounding elements, it becomes evident that DNA fragmentation substantially influences outcomes in Assisted Reproductive Technology (ART). Consequently, this discernible impact has contributed to the integration of sperm DNA fragmentation assessment into advanced evaluations, as endorsed by WHO-2021.
Beyond male age, various other factors exert an influence on DNA fragmentation. These encompass lifestyle components such as smoking and alcohol consumption patterns, exposure to occupational hazards including radiofrequency electromagnetic radiation, environmental pollution, behavioral stress, and

dietary habits, including food processing methods. The upcoming presentation will give particular emphasis to the role of advanced glycation end products (AGEs) and genetic predisposition, particularly focusing on polymorphisms of MTFHR. Additionally, the discussion will delve into the repercussions of excessive supplementation, which can lead to an excessive generation of reactive oxygen species (ROS), a phenomenon known as & quot;reductive stress.& quot; Furthermore, the presentation will explore how oxidative stress and DNA fragmentation could impact the well-being of subsequent generations. Special attention will be dedicated to the connection between these factors and conditions like bipolar disorder, autism, and schizophrenia.

Animal Biotechnology

I-4: Ovum Pick Up in Goats: A New Hormonal Treatment and Semi-Surgery Method

Aghamiri SM[1], Hajian M[2], Jafarpour F[2], Nasr-Esfahani MH[2]

1. Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman
2. Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, Academic Center for Education, Culture and Research
Email: aghamirimorteza@gmail.com, aghamirimorteza@uk.ac.ir

Breeding accelerates by using the genetic predisposition of superior female animals. The production and transfer of embryos and superovulation provide more offspring from valuable female animals. However, different responses to superovulation and a relatively long rest period uterine lavage could decrease the embryo production rate. So, in vitro embryo production has been presented as an attractive alternative to producing more offspring in farm animals. Also, this method can be suitable for animals with problems in uterine placentation or oviducts. In order to maximize the breeding potential, it is necessary to obtain oocytes repeatedly and non-invasively from superior females. Also, a non-aggressive method is required for the collection of oocytes to preserve endangered species. Ovum pick-up (OPU) could be performed outside of the breeding season and even in immature, postpartum, and old animals. The Laparoscopic ovum pick-up (LOPU) is a reliable procedure in small ruminants that supplies the collection of a predictable number of oocytes. Also, this method is less invasive than laparotomy, allowing several collections from one donor. However, it requires an expert team and appropriate instruments. To collect a high number of oocytes, animals must receive gonadotropins. Goats' superovulation protocols commonly contain intravaginal progesterone and multiple porcine FSH injections. Five clinically and paraclinically healthy goats with an average age of 3 ± 0.5 years were selected for this study. Three consecutives of OPU were performed with an interval of one month. A single-injection FSH treatment was used including intravaginal progesterone sponge (ESPONJAVET, HIPRA, Spain) for six days, 0.075 mg cloprostenol (Vetaglandin, Aburaihan, Iran) on day three, and 75 IU Follitropin alpha (Gonal-F, EMD Serono) in day 4.5 (36 h before OPU). A semi-surgical OPU was performed in this study. The ovaries were observed by laparoscopy and exposed from the abdominal wall with forceps. The oocytes were collected from follicles by a 20G needle connecting to a suction device. The in vitro maturation and fertilization were performed in the laboratory and the products were evaluated in every stage. As a result, 146 follicles were aspirated from the ovaries of five goats in three stages. A total number of 139 oocytes were collected. Eighty-one cleaved embryos and 54 blastocysts were observed in the term. In conclusion, the results of this study showed that using single-injection recombinant FSH could replace multiple-injection FSH in the hormonal treatment of OPU in small ruminants. Also, the semi-surgery method could reduce the cost and handling of the OPU procedure.

I-5: Current Status of Embryo Technology Impact on Dairy Cattle Industry in Iran

Niyasari Nasalji A
Faculty of Veterinary Medicine, Department of Theriogenology, University of Tehran, Tehran, Iran
Email: niasarinaslajiamir@gmail.com

Undoubtedly, embryo technology has a great impact on the genetic progress of dairy herds worldwide. The first successful embryo transfer in cattle was performed by Willet et al. of Cornell University, U.S.A. in 1951. They collected embryos from slaughtered cows and transferred them surgically to a recipient. Since then, many developments and modifications have been conducted globally to establish two main approaches (in-vivo and in-vitro) for embryo production in cattle. In-vivo production of embryos relied on superovulation followed by non-surgical embryo recovery and transfer. In-vitro production of embryos relied on the collection of oocytes from ovaries derived from slaughterhouses or via ultrasound-guided transvaginal ovum pick-up. The recovered oocytes were matured and fertilized in-vitro and the putative zygotes were cultured to produce transferable embryos. Both methods have several advantages and disadvantages which need to be considered for national-scale plans. In Iran, embryo transfer in cattle, depending on in-vivo derived embryos, started nearly 40 years ago. This pilot study did not have any particular impact on the dairy cattle industry. In the last 10 years, at least three companies started to produce in-vitro-derived embryos. So far, there is no published data available regarding the number of calves delivered by the latter approach in Iran. We have started in-vivo production of embryos for the last 3.5 years based on superovulation with Iranian human recombinant FSH (Cinnal-f ® , CinnaGen, Iran). Initially, we adjusted the method and the dose of gonadotropins (Khodadadi et al., Theriogenology, 2022, 191: 239-244). In the first large dairy herd with more than 2700 milking cows, we have collected embryos from dairy cows and transferred them to recipient Holstein or cross-bred heifers during winter 2021. Out of 373 embryo transfers, we achieved 219 (58.7%) pregnancies and 190 successful deliveries (50.1%). In the second large dairy farm with about 1500 milking cows, we have concentrated on Holstein heifers as donors and recipients during the mid-spring and summer of 2023. So far, we have transferred both fresh (n=86) and frozen (n=22) embryos with pregnancy rates of 54.6 and 59.1%, respectively. In conclusion, we have developed an affordable and efficient protocol for producing, freezing, and transfer of in-vivo derived embryos. Both dairy farms were very happy about the results and embryo technology had a great impact on the genetic progress of their herds. Our efficient embryo cryopreservation provides a great opportunity for storage and selling embryos nationally and internationally.

I-6: Male Germline Complementation in Chimaeric Sheep

Oback B

Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand
Email: bjorn.oback@agresearch.co.nz

Controlling the male germline drives genetic improvement in livestock breeding. To multiply the male germline, we aimed to produce germ cell-deficient 'host' males and rescue their germline by complementation with wild-type embryonic 'donor' cells.
Cas9 genome editing was used to disrupt key regulators of germ cell development, DAZL or NANOS2, in male ovine fetal fibroblasts via insertion of premature termination codons. Cell strains were isolated by manual selection of mitotic cells, and those with homozygous editing were identified by PCR, sequencing, and TaqI digestion. Validated strains were used as nuclear donors for somatic cell cloning. Testes sections from cloned DAZL-/- and NANOS2-/- neonates showed normal somatic support cells but lacked prospermatogonia. Normal expression was observed for somatic cell-specific transcripts in the testes, confirming that the murine germ cell-deficient phenotype was conserved in sheep.
Next, we tested if the vacant germ cell niche in NANOS2-/- hosts could be filled in early embryonic aggregation chimaeras, using donor blastomeres expressing red fluorescent protein. Overall, four lambs were generated from aggregation blastocysts. By analysing various tissues representative of the three germ layers with droplet digital PCR, the presence of cells from both origins was confirmed in the lamb from day five aggregation, and the others were found to be non-chimaeric. The chimaeric lamb had relatively even contribution of cell lineages across the body and an intact germline, demonstrating that cells from wild-type donor embryos can fill the germline in genetically sterilised male hosts.
Our finding provides a basis for generating chimaeric absolute transmitter rams as an alternative to artificial insemination, potentially increasing the number of offspring from selected males and accelerating genetic gain in extensive farming systems.

I-7: Buffalo Cloning and Genome Editing: Ways for Improved Productivity (Milk and Meat)

Selokar N

Animal Biotechnology Division, ICAR-National Dairy Research Institute, Karnal-132001, India
Email: Naresh.Selokar@icar.gov.in

Domesticated buffaloes contribute significantly to global milk and meat production. In 2019, the global buffalo population of 208 million heads produced 133.75 million tons of milk and 4.2 million tons of beef. In Asian countries such as India, Pakistan, and Bangladesh, the buffalo considers a bovine animal of choice to mitigate the demand for milk and meat. For achieving high productivity per animal, the population of low-producing buffaloes has been bred with high genetic merit bulls. During the last few decades, assisted reproductive technologies (ARTs) such as artificial insemination (AI), embryo transfer (ET), Ovum Pick-Up, and *in vitro* fertilization (OPU-IVF), reproductive cloning (SCNT) have been exploiting at a large scale to increase the population of productive animals. In our laboratory, we are exploring animal cloning technology for faster multiplication of known genotype buffaloes, particularly breeding bulls. Our group produced more than 25 live cloned buffaloes from different donor cell types such as fetal cells, embryonic stem cell-like cells, adult cells, and seminal plasma- and urine-derived cells, and by examination of the growth, fertility, and production of cloned buffaloes and their progeny, there are no differences between clones and non-cloned animals. In addition to buffalo cloning, our group is also exploring the CRISPR genome editing tool kits to produce tailor made buffaloes for the improved productivity. At present, we are attempting to produce the MSTN gene (Myostatin is primarily expressed in skeletal muscle and negatively regulates muscle growth) knockout buffalo for improved meat production and the BLG gene (β-lactoglobulin is a whey protein found in the milk of farm animals, and responsible for milk allergy) knockout for production of hypoallergenic milk. We successfully established pregnancies, and expected to deliver live edited buffaloes before the end of this year. Our research has the potential to contribute to the understanding of buffalo production, genetics, improved milk and meat production, and the development of novel strategies for addressing improved productivity of the buffaloes.

Embryology

I-8: Why Do Euploid Embryos Fail To Implant?

Cimadomo D

Science and Research Manager of GeneraLife Company, IVIRMA Global Research Alliance, Rom, Italy
Email: cimadomo@generapma.it

PGT in experienced clinics allows higher predictivity on blastocyst competence with no impact on the cumulative live birth rate (CLBR) per cycle. As collectively reported by blinded non-selection studies, when full-chromosome non-mosaic aneuploidies are investigated from trophectoderm (TE) biopsies, blastocysts diagnosed euploid may implant in up to 65% of the cases, while aneuploid karyotypes foresee lethality in >98% of the cases. Other than technical errors, euploid-aneuploid mosaicism, whose prevalence is 5%, represents the main limitation of PGT. Yet, attempting at diagnosing it based on intermediate copy numbers (ICN) from a single TE biopsy analysis, does not change the predictive power of dichotomic (euploid/aneuploid) diagnoses. Beyond euploidy, morphokinetics is associated with embryo competence, yet poor morphology (<BB according to Gardner's grading scheme) and day7 development result in non- negligible LBRs (>10%) and are not hallmarks of incompetence. Omics may unveil future biomarkers of competence, but the data must be complemented with more thorough investigations of maternal/paternal characteristics. Moreover, poor or excessive manipulations and some clinical strategies could impact the outcomes in the context of euploid transfers. This talk will summarize all features investigated in the clinical/academic contexts for putative associations with euploid blastocysts' implantation. This knowledge is key to define and implement more efficient (non-)invasive embryo selection tools and clinical workflows in the future.

I-9: Human Wharton's Jelly Hydrogel: A Novel Engineered Bio-material for in Vitro and in Vivo Ovarian Follicle 3d Growth

Fathi R

Department of Embryology, Reproductive Biomedicine Research

Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: rfathi79@gmail.com

I-10: Digitizing The Human Embryo

Shafiee H

Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital and, Harvard Medical School, Boston, Massachusetts, USA
Email: HSHAFIEE@bwh.harvard.edu

Since the first successful birth from *in vitro* fertilisation (IVF) in 1978, advancements in embryo culture systems, micromanipulation, imaging, and genomics have improved the process. However, IVF still faces challenges, as nearly 70% of patients do not achieve live births in the first cycle, and about 30% discontinue treatment. The inefficiencies are attributed to variability in medical practice and the subjective nature of manual embryo grading and visual assessments for critical decision points, such as embryo selection for transfer. Deep learning artificial intelligence (AI) with convolutional neural networks (CNNs) has the potential to revolutionize IVF and assisted reproductive technology. In this talk, we briefly discuss how these AI applications act as a second eye, objectively evaluating embryonic development, tracking embryos throughout the IVF process, and providing morphological assessments to guide clinical decisions.

Female Infertility

I-11: Challenges in PRP Therapy of Poor Responders

Aflatoonian A

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: abbas_aflatoonian@yahoo.com

I-12: How Old is too Late?

Aghahosseini M

Department of Gynecology and Obstetrics, Infertility and IVF Department of Shariati Hospital, Tehran, Iran
Email: marzieh.aghahosseini@gmail.com

To optimize the time to pregnancy, it is essential to recognize the critical role of female age on fertility outcomes. Advanced maternal age typically defined as between 35-38 years, is significantly correlated with diminished fertility potential. This challenge remains unresolved even with the use of assisted reproductive technologies (ART). The underlying reasons for this decline include a reduction in the number of competent oocytes, an increase in aneuploidy, and a heightened rate of embryo arrest. Notably, these complications amplify post 38 years of age. Consequently, oocyte banking in the late 30s may not offer significant benefits for patients. Comprehensive consultation on age dependent fertility is imperative. Surveys indicate a prevalent lack of awareness among women regarding the impact of age on fertility, with many mistakenly believing that regular menstruation cycles signify unproblematic fertility.

I-13: Progesterone Levels and Embryo Transfer in Fresh Embryo: Pregnancy Outcome

Aleyasin A

Department of Obstetrics and Gynecology, Fellowship of Infertility, Tehran University of Medical Sciences, Tehran, Iran
Email: Ivfshariati69@gmail.com

During assisted reproductive technology (ART) cycles, elevated progesterone levels (P> 0.9 ng/ml) on the day of HCG injection can diminish endometrial receptivity. This subsequently leads to notable reductions in both the implantation rate (IR) and ongoing pregnancy rate (OPR), especially in patients with diminished ovarian reserve (DOR). Elevated progesterone can compromise embryo quality, necessitating treatment like cycle cancellation and the " freeze all & quot; strategy. The influence of progesterone is also evident in frozen embryo transfer (FET) cycles, particularly when hormone replacement therapy (HRT) transfer are associated with reduced pregnancy outcomes, while levels exceeding 20ng/ml also present adverse effects.

I-14: Luteal Phase Support

Esmaeilzadeh S

Infertility and Reproductive Health Research Center Health Research Institute, Babol University of Medical Science, Babol, Iran
Email: sesmael2010@gmail.com

Normal luteal function is the main component for pregnancy maintenance. In natural ovulatory cycles, the corpus luteum can produce adequate progesterone after ovulation until the placental function starts at seven wk. gestation. Luteal-phase deficiency is a condition where there is insufficient endogenous progesterone for embryo implantation, which can be associated with infertility and pregnancy loss. LPD is post-ovulatory luteal dysplasia, producing too little progesterone or premature luteal decline, resulting in reduced endometrial secretory responsiveness; the clinical manifestation is asynchronous endometrial and embryonic development, closely associated with infertility or miscarriage.
Luteal-phase support (LPS) is a well-known intervention for almost all stimulated assisted reproductive technology (ART) cycles. Ovarian stimulation cycles using both gonadotropin-releasing hormone (GnRH) agonist or antagonist protocols have been associated with a defective luteal phase that can disturb embryo implantation. Multiple follicular development results in supraphysiological levels of estradiol and progesterone that have negative feedback on luteinizing hormone (LH) secretion from the pituitary gland. Other factors include the disruption of granulosa cell function after oocyte pick up, prolonged suppression of the pituitary after administration of GnRH agonist or GnRH antagonist, and the negative feedback of exogenous human chorionic gonadotropin (HCG) on the secretion of LH from the pituitary.
Very different regimens are suggested for supporting the luteal phase in fresh and frozen-thawed cycles; A: The luteal phase support for fresh transfer cycle include: 1. HCG triggered (Progesterone (IM, Vaginal SC, Oral)), HCG, Estradiol, GnRH-a and 2. GnRH-a-triggered (Dual trigger with low dose HCG and GnRH-a, Low-dose HCG at the time of ovum pick-up, Low-dose HCG in the luteal phase, daily low dose recombinant HCG

in the luteal phase, Luteal coasting, Recombinant LH, Intensive luteal support (steroid-only luteal phase supplementation).
B: LPS for FET: Cycles Protocols used for frozen cycles consist of a natural cycle (NC), a modified natural cycle, and an artificial cycle (AC). Synchronization between the embryo and endometrium is a key factor for successful implantation in FET cycles. The “window of implantation” is the ideal opportunity for trophoblast-endometrial cross-talking; it occurs around days 22-24 of a 28-day cycle.
Knowing the appropriate time for starting LPS is currently challenging. The ESHRE guidelines recommended that LPS should be started in the interval time between the evening of the day of ovum pick up and day 3 post oocyte retrieval.
Duration of luteal support based on available evidence recommends that continuing progesterone products after the first positive pregnancy test is not necessary.
Current national guidelines recommend that progesterone support during assisted reproduction be administered orally in combination with vaginal administration/injections until 10 - 12 weeks of gestation. The dosage should be reduced and discontinued according to the patient's individual circumstances. However, the best luteal support regimen should be selected on a patient-specific basis.

I-15: Controlled Ovarian Hyperstimulation (COH) in ART

Farzadi L

Department of Obstetrics and Gynecology, School of Medicine, Tabriz University of Medical Sciences, Tehran, Iran
Email: royaninstitutecongress@gmail.com

I-15: Fertility Preservation in Female Cancer Patients: Experience of Royan Institute

Ghaffari F

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

Despite the decades-long history of fertility preservation in cancer patients in the world, this field is about a decade old in Iran, and royan institute is the most important active institution in the field. Since 2014, Royan Institute provides comprehensive services of fertility preservation to cancer patients in a multidisciplinary team. The gravity of the issue has led to the formation of the “Oncofertility Taskforce” within this institute in 2017. The taskforce uses the world’s latest, cutting-edge fertility preservation methods, including the oophoropexy, and the freezing of embryos, eggs, sperm, and ovarian and testicular tissue, to preserve fertility in adult women and men and immature girls and boys. It also pursues the mission of conducting relevant research projects aiming to improve the current treatment methods. The members of specialized taskforce are divided into the clinical and research subgroups including the reproductive endocrinologist and urologist, oncologist, perinatologist, advanced laparoscopic surgeon, psychologist, embryologist, anesthesiologist, pathologist, radiologist, genetic, ethics and forensic specialist, epidemiologist, stem cell & developmental biologist, and coordinator and clinical research midwife. The total of cancer patients referred to royan institute since 2014: 417 patients (oocyte cryopreservation: 167 patients, embryo cryopreservation: 89 patients, oocyte and embryo cryopreservation: 40 patients, and ovarian transposition: 2 patients). So far, about 10 live births have been recorded from oocytes and/or embryos cryopreservation. Almost 100 ovarian tissue cryopreservation and two transplantations have been performed for cancer patients in royan institute since 2010.

I-16: Preparation Methods of Endometrium in Freezed Embryo

Hosseini S

Departmenr of Obstetric ans Gynecology, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: hosseinibaran@gmail.com

Conventionally, *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments consist of a fresh embryo transfer directly a Ier ovarian hyperstimulation, which is used in order to retrieve oocytes in the IVF/ICSI procedure. In the conventional IVF/ICSI treatment fresh embryo transfer is possibly followed by one or more frozen embryo transfers in subsequent cycles when enough embryos are available. In the "freeze all" strategy all embryos are frozen to be transferred at later time point when the ovaries are not stimulated. Therefore, this method could reduce the risk of ovarian hyperstimulation syndrome (OHSS, an overreaction to fertility drugs) as OHSS is more severe when pregnancy occurs. Furthermore, studies have suggested that a woman's hormonal response to fertility drugs could affect the lining of the womb making it difficult for an embryo to implant. Thus, it could be beneficial to freeze the embryos and transfer them later when the lining of the womb is not affected by fertility drugs. In the past decade, an increasing number of clinics have applied the 'freeze all' strategy as a standard treatment strategy in their practice. In practice, the 'freeze all' strategy and the conventional strategy can vary technically.

I-17: Fertility Preservation in Gynecologic Cancers

Jafarabadi M

Reproductive Health Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: minajaf@yahoo.com

To preserve the full range of options, fertility preservation approaches should be discussed as early as possible, before treatment starts. Another discussion and/or referral may be necessary when the patient returns for follow-up after completion of therapy and/or if pregnancy is being considered. The discussions should be documented in the medical record.
All oncologic health care providers should be prepared to discuss infertility as a potential risk of therapy.
Semen or oocyte cryopreservation for postpubertal children is suggested with patient assent and parent or guardian consent. For prepubertal children, the only fertility preservation options are ovarian and testicular cryopreservation, which are investigational.
Oocyte harvesting for the purpose of oocyte or embryo cryopreservation is now possible on a cycle day–independent schedule. In estrogen-sensitive breast and gynecologic malignancies aromatase inhibitor-based stimulation protocols are now well-established.
Ovarian transposition (oophoropexy) can be offered when pel-

vic irradiation is performed as cancer treatment. However, because of radiation scatter, ovaries are not always protected, and patients should be aware that this technique is not always successful. Because of the risk of remigration of the ovaries, this procedure should be performed as close to the time of radiation treatment as possible.
It has been suggested that radical trachelectomy (surgical removal of the uterine cervix) should be restricted to stage IA2 to IB cervical cancer with diameter < 2 cm and invasion < 10 mm. In the treatment of other gynecologic malignancies, interventions to spare fertility have generally centered on doing less radical surgery with the intent of sparing the reproductive organs as much as possible. Ovarian cystectomy can be performed for early-stage ovarian cancer.
When is not feasible, and in the setting of young women with breast cancer, GnRHa may be offered to patients in the hope of reducing the likelihood of chemotherapy-induced ovarian insufficiency. Ovarian tissue cryopreservation for the purpose of future transplantation does not require ovarian stimulation and can be performed immediately. In addition, it does not require sexual maturity and hence may be the only method available in children. Finally, this method may also restore global ovarian function. Further investigation is needed to confirm whether it is safe in patients with leukemias. Ovarian tissue cryopreservation is already considered non-experimental in some countries and its experimental status is undergoing evaluation in the United States.

I-18: Impact of BRCA Gene Variant on Oncofertility Counseling

Lambertini M

Department of Internal Medicine and Medical Specialties (DiMI), School of Medicine, University of Genova, Genova, Italy
Email: Matteo.lambertini@unige.it

I-19: Endometrial Preparation for Embryo Transfer

Madani T

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

To decrease time to pregnancy in ART Cycle, the predictive model is suggested and one of these models is belong to Dehillon which account for female age, BMI, cause of infertility, previous live birth and miscarriage, AFC and duration of infertility. For embryo transfer the recent ESHRE guideline ESET is better than DET. For increased pregnancy, different Embryo transfer like double embryo transfer, mix embryo transfer and transfer of blastocyst instead of cleavage suggested. For increased chance of pregnancy, use of ERA or injury, PRP of endometrium and injection of hCG within endometrium before embryo transfer suggested Infertility and breast diseases.

I-20: Role of Biological and Environmental Aspects on Female Fertility (Pregnancy)

Madani T

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

Time to pregnancy is influenced by biological factors: age of couple is one of the biological factors which is very important because in female by advancing age the chance of DNA damage in oocyte increase and in male by increased in age the chance of fertility decrease. Another factor that influenced the fertility is life style of couple, drug, smoking, diet, stress, caffein, alcohol and endocrine disrupting chemical (EDC) have associated with lower pregnancy and EMF (electromagnetic wave) induce biological and genetic effect, one of the most important physiological systems involved with (EMFS) is genital system and by changing hormone function induce role in reproduction and increase chance of infertility.

I-21: Infertility and Breast Disease

Moini A

Department of Gynecology and Obstetrics, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: ashraf.moieni@gmail.com

I-22: Ethical and Legal Issues in Fertility Preservation for Cancer Patients

Omani Samani R

Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: r.samani@gmail.com

Chemotherapy and radiotherapy as routine cancer treatments can destroy reproductive cells including sperms and oocytes causing secondary infertility. For preventing this complication, there is only one way called fertility preservation (FP) in which, gametes or reproductive issues for future use. Technically, there is less problems about freezing embryo, sperm and oocyte and secondary use of them, but still usage of frozen reproductive tissues including ovary and testes is not promising.
First of all, oncologists are responsible for informing cancer patient about FP and guide them to reproductive centers. This requires complete information about the disease and prognosis for better decision of the patient whether to use or not to use FP. Many physician and oncologists hesitate themselves to provide complete information about the diagnosis and specially survival of the disease. Many of them just inform the patients' families, so, patient is not in a good position to make decision for FP. Some of the physicians don't talk about FP when there is a cancer with poor survival that does not seem to be ethical, because patients have autonomy and must make their own decisions.
Then, some ethical issues are general like: Possibility of transmission of cancer cells to the offspring, low life expectancy after cancer treatment, resource allocation specially with limitation of medical resources, informed consent specially for minors, frozen gamete and embryo disposition, posthumous reproduction and posthumous donation.
There are some ethical issues for each procedure for example for embryo freezing although is the most promising method but can be used for matures, already are couples, the cancer is not too invasive so, the patient has the time for doing one or two

IVF cycle, the cancer is not sensitive to hormones but if so, there are some new ovarian stimulation protocols which are not hormone related. Sperm cryopreservation is used for mature male probably single and oocyte freezing is used for mature women, probably single and the cancer is not too invasive so, the patient has the time for doing one or two IVF cycle. Tissue cryopreservation mostly is used for immature patients or invasive cancers not permitting IVF cycle and is not promising for future conception.
Preventing misguide is a rule for every patient including providing correct information about the possibility of future pregnancy, number of frozen oocytes or embryos needed to be relatively confident about future usage, success rate of IVF, …
Also, special tests are needed for evaluating the ovarian capacity and sperm quality to inform the patient better and preventing any misguide.
There must be a triangle of embryologist, oncologist and gynecologist or urologist sharing for make a good decision about the necessity and the protocol of FP.
Keywords: Assisted Reproduction, Cancer, Fertility Preservation, Oncofertility

I-23: Future of Infertility

Parsanezhad ME

Department of Gynecology and Obstetrics, Shiraz University of Medical Sciences, Shiraz, Iran
Email: parsameb@gmail.com

More women of reproductive age are affected by infertility than by cancer, HTN, DM ….or any other disease. Innovation in the field of human reproduction and infertility is highly challenging due to the much complex biological mechanism that are not easy to manage. The regulatory, legal and ethical issue limitation on treatment, technological problem, and research are another difficult point in this way. The use of IVF has been highly increased recently, due to the late childbearing, which will continue in future. In order to control the increasing demand for ART, and streamline existing processes, innovation is essential. Nowadays and in the era of technology concerning the ART, artificial intelligence is going to be the main stone of infertility management, which promise personalization and, at least, partial automation of IVF technique in the near future. The aim of this lecture is to provide an overview of emerging technologies and summaries the rapidly developing state of the ART innovations in automation, with integration of artificial intelligence, involving the patient treatment pathway, gamete/embryo selection, endometrial evaluation and cryopreservation of gametes/embryos.

I-24: Fresh and Freeze Pregnancy

Salehpour S

IVF Center, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saghar.salehpour2014@gmail.com

I-25: Freeze or Fresh Embryo? Which one is better?

Zadehmodarres Sh

Department of Obstetrics and Gynecology, Shahid Beheshti University of Medical Sciences, Tehran
Email: shahrzad.modarres2014@gmail.com

I-26: Role of Lab in Increasing Pregnancy Rate

Zandieh Z

Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: zahrazandieh5@gmail.com

The major purpose of a couple at the first infertility appointment is to get a healthy baby as soon as possible. There are many baselines and treatment-related factors not associated with the ART laboratory that can influence the success of procedures conducted in the laboratory, including patient medical history, clinical protocol and practice, and the quality and quantity of the gametes provided to the laboratory. Therefore, safety, efficacy, efficiency, medical history and diagnosis should all be considered when evaluating ART laboratory technologies.
The ART laboratory can contribute significantly to reducing the time taken to achieve a healthy delivery through standardized practices and good management of ART lab, using methods for sperm selection, optimal embryo culture environment, well embryo assessment and selection, and cryopreservation methodologies.
In addition to, developing technologies, including Artificial Intelligence has the potential to be used as a promising tool to resolve many longstanding challenges in the field of reproductive medicine, as well as to help embryologist and clinicians to make better decisions and predict the chance of succeed to achieve the ultimate goal of a healthy baby in a shorter time

Genetics

I-27: The State of The ART of Genetic Contribution in Primary Ovarian Insufficiency

Bazrgar M

Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: bazrgar.masood76@gmai.com

Premature ovarian insufficiency (POI), as an important cause of female infertility with a global frequency >3%, defined as the loss of ovarian function before age 40. POI is highly clinically heterogeneous and beyond non-genetic factors, namely iatrogenic and immunity etiologies, genetics is a key player. Chromosomal aberrations and FMR1 gene premutations are the most known genetic contributors in POI. The role of single nucleotide variants (SNVs) in POI is emerging especially by increment of whole exome sequencing (WES) studies. In this talk after sharing our findings in Iranian patients, we will have a look at global findings of WES approaches in POI. I will discuss how stringency in data analyses and publication, like considering consequences in loss-of-function organisms and SNVs tolerance prediction, affect confidence of findings. For overall understanding the role of candidate genes their distribution in signaling pathways such as DNA repair, meiosis and mitochondrial functions will be presented. By comparing genetic findings in POI subcategories, particularly those with primary amenorrhea and familial history of POI, their priority for WES approaches will be highlighted. Despitenumerous can-

didate SNVs in POI, the goal of genomic approaches is finding causal SNVs in proband and early diagnosis of genetic susceptibility to POI in relative or unrelated women, to prevent their infertility.
Keywords: Genetics, Premature Ovarian Insufficiency (POI), Single Nucleotide Variants (SNVs), Whole Exome Sequencing (WES)

I-28: A Non-Invasive Artificial Intelligence Approach for The Prediction of Human Blastocyst Ploidy

Hajirasouliha I

Department of Physiology and Biophysics, Institute for Computational Biomedicine, Weill Cornell Medicine of Cornell University, New York, New York, USA
Email: imh2003@med.cornell.edu

In this talk, I present STORK and STORK-A, non-invasive and automated methods of embryo evaluation that use artificial intelligence to predict embryo ploidy status. Our methods used a dataset of 10 378 embryos that consisted of static images captured at 110 h after intracytoplasmic sperm injection, morphokinetic parameters, blastocyst morphological assessments, maternal age, and ploidy status. Independent and external datasets, Weill Cornell Medicine EmbryoScope+ (WCM-ES+; Weill Cornell Medicine Center of Reproductive Medicine, NY, USA) and IVI Valencia (IVI Valencia, Health Research Institute la Fe, Valencia, Spain) were used to test the generalisability of STORK-A and were compared measuring accuracy and area under the receiver operating characteristic curve (AUC).

I-29: Whole-Genome Sequencing Identifies New Candidate Genes for Nonobstructive Azoospermia

Malcher A

Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland
Email: agnieszka.malcher@igcz.poznan.pl

In recent years, several papers have been published regarding identified genetic variants in men with nonobstructive azoospermia using the whole exome sequencing (WES).
However, the whole exome sequencing (WES) provides a genetic diagnosis in only 25-50% of individuals on the other hand, literature shows that application of whole genome sequencing (WGS) to samples previously screened with WES may provide a conclusive cause in 42%. Although WES improved significantly in the last years, it is outperformed by WGS in terms of genomic coverage.
Here, we used the whole genome sequencing to detect potential causative variants in patients with nonobstructive azoospermia (n=39) including also samples of which mutations in WES were not found (n=6).
WGS using Illumina HiSeq X was performed to detect NOA-associated gene candidates. Variants were annotated using the Ensembl Variant Effect Predictor, utilizing frequencies from gnomAD and other databases to provide clinically relevant information (ClinVar), conservation scores (phyloP), and effect predictions (i.e., MutationTaster). Structural protein modeling was also performed.
Using WGS, we revealed potential NOA-associated SNVs, such as: TKTL1, IGSF1, ZFPM2, VCX3A (novel disease-causing variants), ESX1, TEX13A, FAM47C (previously known genes associated with infertility) and BEND2, BRWD3, MAGEB6, MAP3K15, RBMXL3, and SSX3 genes, which may be involved in spermatogenesis.

I-30: Genetics of Human Asthenozoospermia: From Structural to Functional Defects of the Sperm Flagellum

Toureh A

Institute for Advanced Biosciences, Grenoble, Inserm U1209, Cnrs UMR5309, Université Grenoble Alpes, Team Physiology and Pathophysiology of Sperm cells (PPS)
Email: aminata.toure@inserm.fr

In mammals, sperm fertilization potential relies on efficient progression within the female genital tract to reach and interact with the oocyte. This fundamental property is supported by the flagellum, an evolutionarily conserved organelle that provides the mechanical force for sperm propulsion and motility. As a result, spermatozoa unconditionally require proper assembly, morphology and structure of their flagella. In addition, several maturation events occurring during their journey through the genital tracts are essential to activate their flagellar beating and ultimately confer the fertilization ability. Here we will review data we obtained through our international collaborative network regarding genetic investigation of infertile patients displaying asthenozoospermia due to structural defects of the flagellum (MMAF phenotype). We will also present our recent work on patients with functional asthenozoospermia, which unraveled genes involved in ion-dependent signaling pathways and functional maturation events, providing cues for future developments in terms of therapeuticsof asthenozoospermia and male contraception.

Imaging

I-31: Evaluation of Normal and Abnormal Endometrium

Ahmadi F

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: Dr.ahmadi1390@gmail.com

The integration of endometrium is an important factor in the uterine cavity assessment, and it accounts for one of the most challenging issues in infertility treatment centers since several physiologic and pathologic changes should be ruled out during its assessment.
The first line for assessing the endometrium is ultrasound examination; that is TA ultrasound or TV. The main method is TV since it has more quality compared to the TA. 3-DTV is a proper method for detecting uterine anomalies; not only can it show the transverse and the sagittal views, but it can also show the coronal view save images for further usage. Doppler ultrasound examination provides more information with high quality and accuracy. Hysterosonograghy is a method that depicts lesions of the uterine cavity precisely since the infused liquid detaches the endometrium layers. Apart from mentioned items in the measurement, endometrium outline assessment is of great significance when liquid is infused into the uterus. Additionally, hysteroscopy is considered a diagnostic and therapeutic method

in the case of thin endometrium. For instance, intrauterine adhesion and septum could be treated with hysteroscopy.
Endometrium thickness, which is measured in the thicker part of the endometrium with a mid-sagittal view, is said to be the most important item reported. The two-layer thickness should be reported, and in case that there is intracavitary fluid, the sum of two layers should be mentioned in the report exactly. If the endometrium is thickened asymmetrically, the largest anterior and posterior endometrial thicknesses should also be reported separately. If the endometrial entirety is not clear, a non-measurable endometrium should be reported. When intracavitary pathology is present, the total endometrial thickness including the lesion should be recorded. The volume of intracavitary fluid should be measured using three perpendicular dimensions of intracavitary fluid. Endometrial echogenicity, Endometrium midline, and EMJ should be reported as well.

I-32: Current Strategies to Manage A Thin Endometrium

Ghaffari F

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

Managing patients with thin endometrium still represents a major challenge for clinicians. Endometrium thickness less than 7 mm is the most frequently reported cutoff to define a thin endometrium at the time of final oocyte maturation .The prevalence of thin endometrium varies across published studies: it ranges from 2.4 to 8.5% .Several pathologies like , Asherman syndrome ,history of pelvic radiation ,ovarian stimulation with clomiphene citrate ,postpartum endometritis ,septic abortion , fibroids ,hypothalamic hypogonadism ,Müllerian anomalies ,premature ovarian insufficiency reported . And some time any etiology can be found. Thin endometrium not only implicates lower pregnancy rate but also seems to be associated with adverse perinatal outcomes like miscarriages or abnormal placentation. hysteroscopic evaluation of the uterine cavity should be a priority. the main pathophysiological characteristics of TE include :increased uterine artery blood flow resistance , vascular dysplasia , slow growth of glandular epithelium, low expression of vascular endothelial growth factor .Base on main pathophysiological characteristics of TE the treatment options were classified in four main approaches,(I) “hormonal” treatments,(II) “vascular” treatments (III) “growth factor” treatments (IV) application of stem cell .Hormonal approach includes: adjustment of estradiol administration , low dose priming with human chorionic gonadotropin(hCG) in the follicular phase and administration of GnRH agonists in the luteal phase. High blood flow impedance of uterine radial arteries impairs the growth of the glandular epithelium and decreases blood flow in the endometrium according to this concept “vascular” treatments proposed and several adjuvants like Sildenafil, Aspirin, Pentoxifylline, tocopherol, L-arginine, Neuromuscular electrical stimulation and biofeedback therapy have been studied. Growth factor approaches consist of using Granulocyte colony-stimulating factor and PRP. And finally endometrial regeneration with stem cells have been studied recently. but its efficacy in clinical practice, however, is still limited and this treatment should not be offered outside of rigorous research protocols. There is minimal evidence to support any specific protocols or adjuvants to significantly improve pregnancy outcomes in patients with thin endometrium but at the same time, lack of evidence to favor any of the mentioned approaches does not mean that some of these medical treatments might work in selected particular patients. Physicians must balance the prognosis for patients if they proceed with treatment with a thin endometrium or consider alternative treatments like surrogacy.

I-33: Evaluation of Iatrogenic Endometrial Pathologies

Malek M

Imam Khomeini Hospital, Medical Imaging Center, TUMS, Tehran, Iran
Email: Mahrooz.malek@gmail.com

I-34: Endometrial Myometrial Junction Disturbances and Endometrial Challenges

Raoufi M

Department of Radiology, Shahid Modarres Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: masomeraoufi@gmail.com

Subendometrial lesions, also known as subendometrial cysts or subendometrial diverticula, are abnormal fluid-filled cavities that develop within the endometrium, the inner lining of the uterus. These lesions have been associated with various gynecological conditions and can potentially impact fertility. This abstract aims to provide an overview of the relationship between subendometrial lesions and infertility. Infertility affects a significant number of couples worldwide, and its etiology can be multifactorial. Subendometrial lesions have emerged as a possible contributing factor to infertility in some cases. These lesions may disrupt the normal architecture and function of the endometrium, thereby impairing implantation and subsequent embryo development.
The exact mechanisms by which subendometrial lesions influence fertility remain unclear. However, several hypotheses have been proposed. It is suggested that these lesions may alter blood flow patterns within the endometrium, leading to inadequate perfusion and oxygenation of the uterine lining. Additionally, they may create physical barriers that impede embryo implantation or disrupt the delicate hormonal balance necessary for successful conception. Diagnosing subendometrial lesions typically involves imaging techniques such as transvaginal ultrasound or hysteroscopy. Treatment options vary depending on the size and location of the lesion, as well as its impact on fertility. Conservative management approaches include hormonal therapy or expectant observation for smaller lesions that are not significantly affecting fertility outcomes. Surgical interventions such as hysteroscopic resection or excision may be considered for larger or symptomatic lesions.
While evidence regarding the direct association between subendometrial lesions and infertility is limited, some studies suggest a potential link between these two entities. Further research is needed to elucidate the precise role of subendometrial lesions in infertility and to establish optimal management strategies for affected individuals. In conclusion, subendometrial lesions represent a potential factor contributing to infertility. Understanding their impact on fertility outcomes is crucial for appropriate diagnosis and management. Clinicians should consider the presence of subendometrial lesions in patients experiencing unexplained infertility or recurrent implantation failure, and further investigation may be warranted to guide treatment decisions and improve reproductive outcomes.

Oral Presentation

Andrology

O-1: Association between Blood Plasma BCAAs and Sperm Parameters in Iranian Men with and without Metabolic Syndrome

Esmaeili V[1, 2, 3*], Zendehdel M[2], Alizadeh A[1, 3], Dizavi A[4], Khadem R[4], Vesali S[5], Nahid M[6], Shahverdi AH[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Gyn-Medicum, Center for Reproductive Medicine, Göttingen, Germany
4. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, , Tehran, Iran
6. Massoud Clinical Laboratory, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: The metabolomic approach has recently been used in the assessment of semen quality and male fertility. Additionally, the crucial roles of plasma branched-chain amino acids [(BCAAs; including leucine (Leu), isoleucine (Ile), and valine (Val)] levels in metabolic syndrome (MetS) were reported. However, little information exists about the association between BCAA and semen parameters. Our objective was to explore the association between blood plasma BCAAs and sperm parameters in Iranian men with MetS (MetS+) and without MetS (MetS-).
Materials and Methods: We conducted a cross-sectional study financially supported by National Institute for Medical Research Development (NIMAD; Application No. 995329) on 98 men (age: 25–42 years; MetS+: n=28 and MetS-: n=70) who attended Royan Institute, Tehran, Iran. Semen analysis were performed according to the 5th edition of the WHO guidelines. Anthropometrical and biochemical measurements (fasting) were performed on the same day as the semen analysis. The blood plasma concentrations of BCAA were measured using high performance liquid chromatography (HPLC).
Results: The average variables of anthropometric indicators including weight, waist circumference, hip circumference, waist circumference to height ratio, body fat mass, skeletal muscle, resting metabolism and visceral fat in the MetS+ group were higher than MetS- group (P<0.001). The levels of Ile, Leu, Val and total BCAAs in the MetS+ group were higher than in MetS- group (P<0.001). Interestingly, there was a negative correlation (P<0.001) between Val (r=-0.584) and total BCAAs (r=-0.511) and sperm head defect as well as. Val (r=-0.446) and total BCAAs (r=-0.396) with sperm tail defect (P<0.05).
Conclusion: Our study is the first study to show the association between circulating BCAAs levels and sperm parameters in Iranian men. The results show BCAAs, especially Val negatively correlated with sperm morphology in men with and without MetS which warrants further studies.
Keywords: BCAAs, Blood Plasma, Metabolic Syndrome, Sperm Parameters, Valine

Embryology

O-2: Pyridoxamine Protects Human Granulosa Cells against Advanced Glycation End-Products-Induced Disturbances via Oxidative Stress Modulation

Masjedi F[1*], Mirani M[2], Tabei SMB[3], Nasr-Esfahani MH[4]

1. Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Reproductive Biology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: fatemeh.masjedikand@gmail.com

Background: Ovarian advanced glycation end-products (AGEs) accumulation is associated with the dysfunction of granulosa cells (GCs) via oxidants and antioxidants imbalance. Vitamin B6 derivatives positively affected reproduction. The current study was conducted to elucidate the AGEs effects on oxidative stress signaling pathway in human luteinized mural GCs in the presence or absence of pyridoxamine (PM).
Materials and Methods: Isolated GCs of 50 healthy women were divided into four parts and treated with media alone (control), PM alone, or human glycated albumin (HGA) with/without PM. Total oxidative status (TOS) and total antioxidant capacity (TAC) were assessed by colorimetric methods in GCs lysate. Oxidative stress index (OSI) was also calculated. The AGE receptor (RAGE) protein was also determined using Western blotting.
Results: Non-toxic concentration of HGA significantly increased TOS and OSI, but decreased the TAC levels. The increased RAGE protein expression was also confirmed by western blot analysis. Co-treatment with PM ameliorated the HGA-altered oxidative status and, thereby, corrected the aberrant levels of TOS and TAC. These effects are likely mediated through the regulation of the RAGE protein expression.
Conclusion: This study indicates that oxidative pathway disruption induced by the AGEs-RAGE axis in luteinized GCs are likely rectified by PM treatment. This effect is likely acquired by reduced expression of RAGE protein. A better understanding of how AGEs and PM interact in ovarian physiology and pathology may lead to more targeted therapy for treating ovarian dysfunction.
Keywords: Granulosa Cells, Advanced Glycation End-Products, Pyridoxamine, Oxidative stress

O-3: Interaction of Mouse Blastocyst with The Human Recellularized Endometrial Scaffold

Sadeghi E*, Rezazadeh Valojerdi M, Salehnia M

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

Background: The aim of this study is to evaluate the interaction between mouse embryos as surrogate embryos with the

human endometrial scaffolds that are recellularized with endometrial mesenchymal stem cells.
Materials and Methods: For this purpose, after preparation of the human decellularized endometrial scaffolds they were recellularized by endometrial mesenchymal cells. The adult female mice were super-ovulated by intraperitoneal injections of 7.5 IU of pregnant mare serum gonadotropin followed by 10 IU of human chorionic gonadotropin 48 hours later. The mice were individually put with mature mouse males, then the embryos at the blastocyst stage were collected. The embryos were labeled with Hoechst dye and placed on the top of each scaffold in DMEM/F-12 media for 24 and 48 hours. The implantation and penetration of the mouse embryo into the scaffold were investigated through morphological by light and laser confocal scanning microscope, ultrastructural by scanning electron microscopy, and hormonal study.
Results: Morphological evidence from light microscopy, scanning electron microscopy, and laser scanning confocal microscope (LSCM) indicated that embryos were attached to the surface of the scaffold after 24 hours and penetrated into the scaffold after 48 hours. Also, the further development of the mouse embryo as a cylindrical stage was observed with well-defined epiblast cells. Furthermore, the level of β-hCG was increased during the culture period.
Conclusion: The mouse embryo could be attached and penetrated into the human decellularized scaffold and it could be a good implantation model.
Keywords: Endometrium, Mouse Blastocyst, Implantation

Genetics

O-4: Comparison of Ferroptosis and Autophagy Genes in Menstrual Blood-Derived Mesenchymal Stem Cells between Normal Women and Women with Endometriosis

Hedayatnia H[1, 2*], Kalhor N

1. Department of Biology, Islamic Azad University, Science and Research, Tehran, Iran
2. Department of Mesenchymal Stem Cells, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran
Email: hassanhedayatnia_3@yahoo.com

Background: The etiology of endometriosis is not yet fully understood. Studying how endometriosis develops can help to create better treatments and increase knowledge about the condition and will greatly facilitate its diagnose.
Materials and Methods: In this study, we assessed for the first time the differences in expression levels of some ferroptosis -related genes(GPX4,TXN2,TXNRD1,NRF2) and some autophagy- related genes (ATG7,ATG14,Beclin) between menstrual blood derived stem cells (MenSCs) taken from women with endometriosis and these cells in normal women.
Results: The investigation found that women with endometriosis had upregulated mRNA expression of Beclin-1 (an autophagy-related gene) compared to the normal group ($P \leq 0:024$). No significant difference was found in the expression of other autophagy-related genes (ATG7, ATG14). TXNRD2 (a ferroptosis-related gene often overexpressed in various cancer types) also showed upregulation ($P \leq 0:000$) in women with endometriosis, but no significant difference was detected in the expression of other ferroptosis-related genes (Nrf2, GPX4, TXN2).
Conclusion: We found variations in gene expression levels of ferroptosis and autophagy genes in menstrual blood-derived mesenchymal stem cells (MenSCs) taken from women with endometriosis compared to normal women, suggesting a role in endometriosis development. This supports the retrograde menstruation theory and contributes to Multiomics profiling studies. MenSCs can aid discovery of new drug targets, biomarkers, and non-invasive diagnostic strategies, and predict endometriosis risk in healthy women.
Keywords: Autophagy, Endometriosis, Ferroptosis, Menestrual Blood, Mesenchymal Stem Cells (Mscs)

O-5: A Novel Mutation in The Sun5 Gene in an Infertile Man with Acephalic Spermatozoa Syndrome

Hosseini SH[1*], Salehisedeh N[1], Ahani A[2], Sadighi Gilani MA[1], Sabbaghian M[1]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Mendel Laboratory, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: Acephalic spermatozoa syndrome (ASS) is one of the most severe male spermatogenic disorders defined as a large number of headless spermatozoa in the ejaculate. Although genetic factors play an important role in spermatogenesis, only a few genes definitely were correlated with sperm defects and male infertility.
Materials and Methods: In this regard, whole exome sequencing (WES) was performed on three individuals of this family. PCR reaction, Sanger sequencing, and immunocytochemistry were performed to confirm the results of WES.
Results: We identified a novel homozygous mutation (NM_080675: exon11: c.879dupc: p.k) in the SUN5 gene and the Sanger sequencing approved our obtained result. Additionally, there was not any signal of SUN5-antibody as the results of protein assessment in the spermatozoa of our mutant patient, although we observed a sharp signal of SUN5-antibody in controls.
Conclusion: Our findings suggest that the novel mutation of the SUN5 gene is responsible for ASS. These results will help in the genetic counseling of patients with ASS, on the other hand, according to previous studies, we recommended intracytoplasmic sperm injection (ICSI) to this patient and we are following up the results to complete our findings.
Keywords: Acephalic Spermatozoa Syndrome, Genetic Mutation, SUN5 Gene

O-6: Hub Gene Discovery in Azoospermia

Khoshandam M[1, 2*], Minuchehr Z[2], Kalhor N[3], Karimzadeh M[4], Soltaninejad H[5], Naserpour L[1]

1. Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom branch, Iran
2. Systems Biotechnology Department, Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
3. Core Research Facilities (CRF), Isfahan University of Medical Science, Isfahan, Iran
4. Faculty of Interdisciplinary Science and Technology, Tarbiat Modares University, Tehran, Iran
Email: mohi_khosh70@yahoo.com

Background: Non-occlusive azoospermia (NOA) is an illness related to spermatogenic clutters. As of now, the particular etiologic instrument of NOA is vague. In this study, using bioinformatics analysis, we have investigated key genes and miRNA markers, along with the cellular-molecular pathways in azoospermic individuals.
Materials and Methods: GSE45887, GSE45885, and GSE145467

quality expression profiles were obtained from the gene expression omnibus (GEO) database. DEGs were distinguished between the tissues of homologous occlusive azoospermia (OA) and NOA tissues utilizing the GEO2R device using Venn Diagram, and the common qualities of different communication were screened in two data sets. The Enrichr database was utilized to perform quality cosmology (GO), the kyoto reference book of qualities and genomes (KEGG), and MSigDB Trademark pathways improvement analysis to annotate common DEGs. Using cytoscape, a protein-protein interaction (PPI) organization was created with data collected from dependency genes/proteins (STRING). Cytohubba in Cytoscape was utilized for center quality screening. Besides, the center qualities were approved based on a partitioned dataset. To confirm the bioinformatics results, we took the testicular tissue of a patient with azoospermia with the consent of the patient and the doctor, extracted RNA and synthesized cDNA, and with primers designed for 5 key genes (PTTG1, SPAG5, PLK1, SMAD2, TNFSF10) RT-PCR test we investigated to confirm 5 biomarkers.

Results: 5889 DEGs (3424 upregulated and 2465downregulated) were detected in the GSE145467 dataset, 977 DEGs (114 downregulated and 863 upregulated) were detected in the GSE45885 and 996 DEGs datasets 106 downregulated) was detected in the GSE45887 database After screening with adjusted Pvalue <0.05 and | logFC |> 1.Among DEGs, 590 upregulated genes and 45 downregulated genes were commonly observed in 3 datasets (Figure UP and downregulated). Our laboratory studies through PCR indicated the presence of expression of five genes PTTG1, SPAG5, PLK1, SMAD2, and TNFSF10 in the testicular tissue of men with azoospermia, which were obtained based on bioinformatics results as biomarkers and gene signatures in people with azoospermia.

Conclusion: Our bioinformatic results showed changes in the expression of ten genes as biomarkers in people with azoospermia, and the results of further laboratory investigations confirm the expression of 5 genes in the testicular tissue of a person with azoospermia, although our results need additional tests. As a whole, the discernible evidence of hyper-centric qualities and pathways will help us to provide biomarkers and potential remedial targets for azoospermia.

Keywords: Azoospermia, Biomarkers, Functional Enrichment Analysis, STRING

O-7: Evaluation of IL6, IL6R and Related Micrornas Expression in Endometrium of RIF Patient and Fertile Women Referred to Royan Institute

Takhiri F[1,*], Zavareh S[1], Ghafari F[2], Ghorbanmehr N[3], Jahangiri M[4], Movaghar B[4]

1. School of Biology, Damghan University, Damghan, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, Alzahra University, Tehran, Iran
4. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: b.movaghar@royaninstitute.org

Background: Secretion of hormones and expression of many genes, including inflammatory genes, change during the implantation window. In many cases, the regulation of gene expression is done by microRNAs.To compare the expression IL6 and its receptor (IL6R) as inflammatory factors, as well as their related microRNAs (miR-146a, miR-451 respectively).

Materials and Methods: In this case-control study, endometrium tissue was taken from the uterus of 16 recurrent implantation failure (RIF) and 9 fertile women between days 19-23 of the menstrual cycle at the Royan Institute. Endometrium dating test was carried out using hematoxylin and eosin staining. QPCR was used to investigate IL6 and IL6R and related microRNAs(miR-146a, miR-451). The statistical analysis was done with REST.

Results: The histological examination of RIF samples showed that 7 samples were in the beginning and 9 samples were in the middle of the secretory phase. The expression of IL6 was significantly lower in RIF women(mid secretory) and fertile women compared with RIF women(early secretory, $P<0.05$). IL-6R was not detected in any of the groups. The highest expression of miR-146a was seen in fertile women($P<0.05$). There was no significant difference in miR-451 among the groups.

Conclusion: Considering the embryo transfer timing challenge in RIF patient and difference in miR-146a between RIF and fertile women, it may be possible to use it as a biomarker to determine the exact time of window of implantation (WOI) in RIF patient individually. In the next step, it is better to consider larger number of samples.

Keywords: IL6, Inflammation, Repeated Implantation Failure, miR-146a

O-8: Engineered Exosome as A Biological Carrier for Encapsulation of Rosmarinic Acid to Enhance Implantation Rate in Mice with Induced Endometritis

Taravat M[1*], Asadpour R[1], Fattahi A[2], Hajipour H[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: morteza.taravat@tabrizu.ac.ir

Background: Endometritis is a histopathologic condition that affects the proper decidualization and implantation processes of the embryo. In this study, exosome-containing Rosmarinic acid (RA) was used to dampen lipopolysaccharide (LPS)-induced endometritis and subsequently enhance implantation.

Materials and Methods: In this study, exosomes extracted from the serum samples were loaded with RA acid and then administrated into the animal groups, including RA, exosome, RA plus exosome (RA+Exo), and RA-loaded exosomes (RALExo) groups. The concentrations of RA or exosomes used in this study was 10 mg/kg, and the compounds were injected into the uterine horn 24 hours following the induction of endometritis. Upon the presence of inflammation detected by the histopathological method, the most proper groups were mated with male mice. The effect of the treatment group on the implantation rate, progesterone levels, and gene expression was assessed by Chicago Blue staining, ELISA, and RT-PCR, respectively.

Results: According to the obtained results, RALExo10 and RA10+Exo10 groups exhibited improved pathological alterations, enhanced progesterone levels, increased implantation rate, as well as heightened expression levels of LIF and Muc-16 genes. Besides, the expression levels of inflammatory cytokines, including TGF-β and IL-10, IL-15, and IL-18, were regulated.

Conclusion: Our findings indicated that the expression of LIF, Muc-16 genes as well as IL-18, were significantly correlated with serum progesterone concentrations and the implantation rate in the treatment groups.

Keywords: Exosome, Implantation, Endometritis, Rosmarinic Acid

Poster Presentation

Andrology

P-1: Evaluation of Silymarin Protective Potential in Testicular Histoarchitecture Following Ischemia/Reperfusion in Mouse

Aghaei F[1*], Shahrooz R[1], Ahmadi A[1], Mazaheri Khamene R[2]

1. Department of Basic Veterinary 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-detorsion (TD) is a testicular ischemia-reperfusion (I/R) process that can cause various morphological changes in the testicular tissue and lead to reduced fertility or infertility. Silymarin is a polyphenolic flavonoid that known as an antioxidant and anti-inflammatory agent with several therapeutic impacts. The aim of this study was to evaluate the protective potential of silymarin on the histological changes of the testis in mice that are affected by unilateral testicular torsion-detorsion.
Materials and Methods: 32 adult male NMRI mice were randomly divided into four groups. Group I: control sham, Group II: silymarin, without testicular torsion in these two groups, Group III: ischemia-reperfusion (I/R) and Group IV: I/R plus silymarin (I/R +S). The testicular torsion was performed by rotating the 720 ° spermatic cord the left testis in a counterclockwise direction. After 1 hour, with a rotation opposite to the previous direction, detorsion was done. After the derision, the animals received 50 mg / kg of silymarin via gavage for 35 days. At the end of experiment period, all of the left testicles were removed. Following tissue preparation processes and H&E staining, the histomorphometrical studies were performed. Data were analyzed by ANOVA and post hoc Tukey test ($P < 0.05$).
Results: The diameter of seminiferous tubules, the thickness of germinal epithelium and the cross-sectional area were decreased in the I/R group compared to other groups ($P<0.05$) and the thickness of testicular capsule increased in the I/R group compared to other groups ($P<0.05$), while a significant amelioration was observed in the group received silymarin (I/R+S) compared to the I/R group.
Conclusion: It is concluded that treatment with silymarin reduces adverse histomorphometrical changes caused by experimental unilateral testicular ischemia/reperfusion in the testicular tissue of mice. This may be due to the antioxidant effects of silymarin.
Keywords: Ischemia, Reperfusion, Silymarin, Testis

P-2: Assessing Sperm DNA Damage: A Comparison between Oligozoospermic and Normozoospermic Infertile Men

Arbabian M[1*], Tavalaee M[1], Behdarvandiyan P[1], Nasresfahani A[2], Pashaei K[2], Nasr-Esfahani MH[1, 2]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: maryam.arbabian@yahoo.com

Background: Oligozoospermia, characterized by reduced sperm concentration, is often indicative of poor sperm motility and morphology, resulting in abnormalities in spermatogenesis within the testis. Several studies have demonstrated that sperm DNA integrity plays a role in successful clinical outcomes. Therefore, we evaluated sperm DNA in a large population of infertile men with oligozoospermia and normozoospermia.
Materials and Methods: This retrospective cohort study included 500 samples from oligozoospermic patients whose sperm count was 39 million per ejaculate and 500 samples from normozoospermic men with sperm count≥39 million per ejaculate, according to the World Health Organization criteria. In addition, sperm DNA damage was assessed using the SCSA and TUNEL assays. To compare study parameters between the two groups, we used an independent sample t-test. Statistical significance was set at $P < 0.05$.
Results: The results indicate that There were no significant differences in age and body mass index between oligozoospermic and normozoospermic men. However, oligozoospermic men had significantly lower mean values of sperm motility, progressive motility, and sperm count than normozoospermic men ($p<0.001$). Additionally, oligozoospermic men had significantly higher mean values of abnormal sperm morphology, sperm DNA damage assessed by SCSA, and TUNEL assays than normozoospermic men ($p<0.001$).
Conclusion: Oligozoospermia is associated with impaired sperm quality and DNA integrity and may lead to male infertility. Further research is needed to develop effective treatments for oligozoospermia.
Keywords: Normozoospermia, Oligozoospermia, Sperm DNA Damage, Sperm Parameters

P-3: Resistance Exercise Alone and with Herbal Supplements Can Improve The Sperm Parameter in Infertile Men with Oligo-Astheno-Teratozoospermia: A Randomized Single Blind

Asa E[1*], Delshad A[1, 2], Fazaeli[1, 3], Salami F[1, 4], Verdi A[1], Naserpour L[1], Azadpoor M[1, 5]

1. Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran
2. Department of Sports Physiology and Immunology, Faculty of Literature and Human Sciences, University of Qom, Qom, Iran
3. Department of Mesenchymal Stem Cells, Academic Center for Education, Culture, and Research(ACECR), Qom, Iran
4. Department of Sports Sciences, Faculty of Literature and Humanities, University of Qom, Qom, Iran
5. Andrology Ward, Infertility Treatment Center, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran
Email: asa.elham@gmail.com

Background: We aimed to investigating the effects of resistance exercises with palm pollen or ginger supplements on sperm parameters, chromosome breakage, and sex hormones on infertile men with oligoasthenoteratozoospermia.
Materials and Methods: This randomized single-blind, placebo-controlled trial was performed on 48 infertile men with oligoasthenoteratozoospermia who were admitted to in the unit of the Infertility Research Center of the Academic Center for Education, Culture and Research (ACECR) (Qom, Iran). Par-

ticipants were randomly divided into 6 groups of resistance training (n=8), palm pollen (n=8), ginger (n=8), resistance training + palm pollen (n=8), resistance training + ginger (n=8) and control (placebo) (n=8). The participants in the supplement and supplement with exercise groups took 500 mg of ginger supplement and palm pollen supplement at a dose of 100 mg during a two-month period. Before and after therapy, the semen and blood sample were collected.
Results: The statistical analysis of the data showed that the motility in the supplement and the supplement groups with exercise was increased significantly compared to before the treatment (P<0.5). motility and sperm concentration in the supplement group showed a statistically significant increase (P<0.5), the rate of DFI showed a significant decrease in all the studied groups except the control group (P<0.5). Also, the level of the studied hormones in all the groups significant increase was observed except the control group.
Conclusion: Considering that the sperm parameters improved after consuming palm pollen and ginger supplements alone and with exercise compared to the control group.
Keywords: Ginger, Oligoasthenozoospermia, Palm Pollen, Resistance Exercise, Sperm Parameteres

P-4: *In Vitro* Effect of Alpha-Lipoic Acid on Motility, Acrosome Reaction and Membrane Integrity of Human Sperm

Atshan[1*], Soleimani Mehranjani M[1], Shariatzadeh Seyed MA[1], Cheraghi E[2]

1. Department of Biology, Arak University, Arak, Iran
2. Department of Biology, Qom University, Qom, Iran
Email: Roksana.atshan@yahoo.com

Background: ALA Alpha lipoic acid (ALA) is a cofactor of several enzyme complexes in the mitochondria and has antioxidant properties. The effect of ALA on motility, acrosome reaction and membrane integrity of human sperm was evaluated *in vitro*.
Materials and Methods: Semen samples of 30 fertile men were divided into: fresh, control and ALA (0.02mM ALA for one hour). Sperm motility was evaluated with light microscope, sperm membrane integrity with hypo-osmotic swelling (HOS) test and acrosome reaction with Fluorescein isothiocyanate-labelled pisum sativum agglutinin (FITC-PSA) staining. Data were analyzed using Repeated Measure analysis.
Results: A significant increase (P<0.001) in the total motility and the progressive sperm motility was observed in the ALA group compared to the fresh and control groups (P<0.001). While, a significant decrease (P<0.05) in the mean non-progressive motility was reported in the ALA group compared to the fresh and control groups. The mean integrity of the sperm membrane in the ALA group showed a significant increase compared to the control group (P<0.001). A significant decrease in the mean percentage of premature acrosomal reaction was observed in the ALA group compared to the control group (P<0.001).
Conclusion: ALA prevents membrane destruction and premature acrosomal reaction due to its antioxidant properties and also increases sperm motility.
Keywords: Antioxidant, Infertility, Sperm

P-5: Effect of Paroxetine on Progressive Motility, Mitochondrial Membrane Potential and Sperm DNA Fragmentation: A *In Vitro* Study

Atshan M[1*], Soleimani Mehranjani M[1], Shariatzadeh Seyed MA[1], Cheraghi E[2]

1. Department of Biology, Arak University, Arak, Iran
2. Department of Biology, Qom university, Qom, Iran
Email: Roksana.atshan@yahoo.com

Background: Paroxetine causes oxidative stress in various cells by destroying mitochondria. So far, there has been no study related to the direct effect of paroxetine on human sperm. The aim of this study is to investigate the effect of paroxetine on progressive motility, mitochondrial membrane potential (MMP) and DNA fragmentation of human sperm *in vitro*.
Materials and Methods: The semen samples of 30 fertile men were divided into three groups: the fresh group, the control group and the group treated with 5 µM paroxetine for one hour. Sperm progressive motility was evaluated by light microscopy, MMP by rhodamine 123 staining and sperm DNA fragmentation by acridine orange staining. Data was analyzed using Repeated Measure analysis.
Results: A significant decrease in the mean progressive sperm motility and the mean MMP was observed in the paroxetine group compared to the fresh group and the control group (P<0.001). Meanwhile, A significant increase (P<0.001) in the mean percentage of sperm DNA fragmentation was reported in the paroxetine group compared to the fresh group and control.
Conclusion: It seems that paroxetine induces oxidative stress causing damage to the mitochondrial membrane and thus leading to a decrease in progressive motility and an increase in sperm DNA fragmentation.
Keywords: Infertility, Paroxetine, Sperm

P-6: Corniculatusin and Prostate Cancer: A Comprehensive Network Pharmacology Study

Bahavar H[1, 2*], Kaveh Vernousfaderani E[1, 2], Jabbarzadeh A[1, 2 2], Farhadi Nejad K[1, 2], Farnam G[3], Shirazi F[2, 3]

1. PharmS, Student Research Committee, Pharmaceutical Sciences Research Center, Tehran, Iran
2. Pharmacology and Toxicology Department, School of Pharmacy, Tehran, Iran
3. Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: F.shirazi@sbmu.ac.ir

Background: Prostate cancer is the second most common male cancer diagnosis and fifth lethal cancer around the world in 2020. There is no evidence yet on how to prevent prostate cancer; however, the risk can be lowered down by reducing high-fat foods consumption, following a regimen high in vegetables and fruits and becoming more involved with exercise. Evidences confirm that there is a correlation between male infertility and later PC. Throughout history, Plants have been used as agents in cancers' therapies. Flavonols, are second metabolites of plants, found in diverse plants. Studies confirmed flavonoids' effects as anti-inflammatory, antioxidant, antibacterial and anticancer. Corniculatusin, also known as 3,3',4',5,7-pentahydroxy-8-methoxyflavone, is a pentahydroxy flavone, found in Sedum alpestre and S. apoleipon.
Materials and Methods: In this study we utilized various databases such as PubChem, Binding DB, SwissTargetPrediction, SEA, TargetNet, GeneCards, and DisGeNET to identify PC-related targets of corniculatuisn. The STRING database was used

to evaluate protein-protein interactions and DAVID to calculate the gene ontology of these proteins. The network pharmacology diagram was depicted using Cytoscape 3.9.1.
Results: Related to the gene ontology, positive regulation of transcription from RNA polymerase II promoter, nucleus, and enzyme binding are the most probable processes affected by corniculatusin. Glycogen Synthase Kinase 3 Beta (GSK-3β) and AR (Androgen Receptor) are the most influenced targets, according to network pharmacology diagram. Mutations of androgen receptors (AR) may be the reason of some male infertility cases due to researches which depicted the role of AR signaling in spermatogenesis in the maintenance of spermatogonial numbers, blood-testis barrier integrity, completion of meiosis, adhesion of spermatids and spermiation.
Conclusion: Results of network pharmacology studies and scrutiny of the databases showed that corniculatusin would have the most impact on the CWR22R cell line. Moreover, corniculatusin appears to be an effective agent against multiple prostate cancer stages with various pathways leading to tumor suppression and metastasis.
Keywords: Corniculatusin, Network Pharmacology, Prostate Cancer, Sedum Alpestre

P-7: 3,4-Methylenedioxymethamphetamine Effects on The Male Fertility Capacity

Bayat H , Ghasemi Y , Movassaghi Sh , Nadia Sharifi Z

Department of Anatomical Sciences & Cognitive Neuroscience, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
Email: shmovassaghi@iautmu.ac.ir

Background: Ecstasy or 3, 4 Methylene dioxy MethAmphetamine (MDMA) is an addictive and hallucinogenic chemical compound. Studies have shown MDMA with increasing production of oxidative stress can have negative effects on male reproductive capability. This study aimed to investigate the effect of MDMA administration on the testicular interstitial tissue of Wistar rats.
Materials and Methods: In this study, 18 adults male Wistar rats (weight: 250-300 g) were randomly divided into 3 groups: MDMA group (receiving 7.5 mg/kg MDMA 3 times every 2 hours for one day), vehicle group (injection of normal saline) and control group (without any intervention). The animals were sacrificed 2 weeks later and their testicles were H&E staining and the number of viable Leydig cells counted.
Results: There was a significant decrease in the number of Leydig cells in the MDMA group compared with the control and vehicle groups ($P \leq 0.05$). But There was no significant difference between the number of viable Leydig cells in control and vehicle groups ($P > 0.05$).
Conclusion: The results of this study showed that the using of Ecstasy can be damaged normal homeostasis of the testis by reducing cell proliferation in the interstitial tissue of the testis.
Keywords: Leydig Cells, Male Infertility, Methamphetamine, Testis, Rat

P-8: Circulating Branched Chain Amino Acids Levels of Men with Different Body Mass Index Are Associated with Sperm Parameters

Esmaeili V[1, 2*], Zendehdel M[2], Alizadeh A[1, 3], Dizavi A[4], Khadem R[4], Vesali S[5], Nahid M[6], Shahverdi AH[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, , Royan Institute for Reproductive Biomedicine, ACECR, , Tehran, Iran
2. Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Gyn-Medicum, Center for Reproductive Medicine, Göttingen, Germany
4. Department of Andrology, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
6. Massoud Clinical Laboratory, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: Overweight and obesity have negative effects on male fertility. Although several recent studies provide evidence regarding circulating total branched-chain amino acid (BCAA) levels (leucine (Leu), isoleucine (Ile), and valine (Val)) are elevated in obesity and diabetes. Our purpose was to investigate the assessment of circulating BCAAs levels and sperm parameters in men with different BMI.
Materials and Methods: This cross-sectional study financially supported by National Institute for Medical Research Development (NIMAD; Application No. 995329) was conducted financially supported by National Institute for Medical Research Development (NIMAD; Application No. 995329) on 98 men in three groups: normal-weight (Nw; body mass index: BMI<24.9 kg/m^2), overweight (Ow; BMI:25–29.9 kg/m^2), and obese (Ob; BMI:30–35 kg/m^2). Semen analysis were performed according to the WHO guidelines. Anthropometrical obesity-related markers and fasting biochemical parameters were performed on the same day as the semen analysis. The blood plasma concentrations of BCAAs were measured using high performance liquid chromatography (HPLC).
Results: The mean waist circumference, hip circumference, waist circumference to height ratio, body fat mass, skeletal muscle, resting metabolism and visceral fat were remarkably higher for subjects in the Ob group than in Ow and Nw ($P<0.001$). The Ob group had significantly lower sperm total motility, progressive motility and viability than the Nw groups ($P<0.05$). In terms of semen volume, sperm count, and sperm head and tail defect, there was no significant ($P>0.05$) difference between the groups. Interestingly, Val and total BCAAs were significantly higher in the Ob group than in Ow and Nw groups ($P<0.05$). Uniquely, there was a strong significant negative correlation between Val ($r=-0.639$) and total BCAA ($r=-0.491$) with sperm head defects in Ob group ($P<0.05$).
Conclusion: Our findings in the Ow and Ob groups support a negative relationship between blood plasma BCAAs levels; especially Val and sperm parameters. Therefore, the key role of Val among BCAAs family members and the negative effect of higher Val on sperm parameters and male fertility warrants further studies.
Keywords: BCAAs, Blood Plasma, BMI, Sperm Parameters, Obesity

P-9: Effectiveness of Varicocelectomy in Improving Sperm Parameters and Functional Tests in Infertile Men with Varicocele: A Comparative Study

Esmaeili Irani A[1*], Arbabian M[1], GHorban Z[1], Tavalaee M[1], Nasr-Esfahani MH[1, 2]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: asi1997.ae@gmail.com

Background: Our study aimed to evaluate the effects of varicocelectomy, a common treatment for clinical varicocele-related male infertility, on sperm parameters and functional tests. To achieve this, we conducted a comparative analysis of pre- and post-surgery results.
Materials and Methods: Our study recruited 100 infertile men with grade II and III varicocele who were scheduled for varicocelectomy. We collected semen samples from all participants before the surgery and three months post-surgery to assess changes in semen parameters using a computer- assisted sperm analysis (CASA) system. We also evaluated oxidative stress, histone residual, protamine deficiency, and DNA damage using bodypi, aniline blue, chromomycin A3, and acridine orange staining, respectively. We conducted statistical analysis using a paired t-test to compare the study parameters between the two groups, with statistical significance defined as a p-value of less than 0.05.
Results: Our study demonstrated that varicocelectomy resulted in significant improvements in sperm concentration, morphology, and motility ($P<0.01$) compared to pre-surgery values. Additionally, we observed significant reductions in oxidative stress, histone residual, protamine deficiency, and DNA damage ($P<0.05$) after varicocelectomy compared to before the surgery.
Conclusion: Our study confirms that varicocelectomy is effective in improving sperm parameters, reducing oxidative stress, and improving sperm chromatin packaging and DNA damage in infertile men with varicocele. Notably, our study benefits from a large sample size and a consistent surgical approach, as all surgeries were performed by one surgeon using microsurgery. These findings highlight the potential benefits of varicocelectomy as a treatment option for male infertility related to varicocele.
Keywords: DNA Damage, Oxidative Stress, Sperm Parameters, Varicocelectomy, Protamine Deficiency

P-10: Protective Effects of Curcumin on Body and Testis Weight, and Testosterone Levels after Chronic Acrylamide Exposure in Mice

Ghane F*, Ahmadi A, Imani M, Shalizar Jalali A

Department of Basic Sciences, , Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: Fahimekh685@yahoo.com

Background: Acrylamide has been shown to have reproductive toxicity. The aim of this study is to evaluate the protective effects of Curcumin following long-term exposure of Acrylamide on body and testis weight and serum testosterone levels.
Materials and Methods: Forty male mice (age: 6-8 weeks) were divided into five groups. The control group received normal saline (0.2ml/day), Acrylamide treated group (50mg/kg, 0.2ml/day), the first and second experimental group received Curcumin (100 and 200 mg/kg respectively, 0.2 ml/day) one hour before Acrylamide (50mg/kg, 0.2ml/day) and Curcumin group received (200 mg/kg, 0.2ml/day) orally for 45 days. Animal weight and their testicles were measured, and blood samples were taken to prepare serum. Serum testosterone levels was measured by ELISA. Statistical analysis was done by SPSS software, One Way ANOVA (Tukey) test ($P<0.05$).
Results: A significant decrease in average of weight difference between the beginning and the end of the treatment, the average of weight of the right and left testes and serum testosterone levels was observed in Acrylamide treated group compared with the control group ($P<0.05$). A significant increase in average of the body weight difference and serum testosterone levels was observed in second experimental group and Curcumin group compared with the Acrylamide treated group ($P<0.05$). Increasing of average of the testes weight in the experimental groups was not significant compared with the Acrylamide treated group ($P<0.05$).
Conclusion: Chronic Acrylamide exposure could decrease body and testes weight, and serum testosterone levels by inducing oxidative stress, and oral administration of Curcumin (200mg/kg) along with Acrylamide could neutralize these effects of Acrylamide and prevent reproductive toxicity.
Keywords: Acrylamide, Curcumin, Mice, Testis, Testosterone

P-11: Investigating The Impact of Doxepin Hydrochloride on The Process of Spermatogenesis

Golian H[1*], Nasri S[2], Mirdar-Soltani M[1, 3]

1. Depatmant of Biotechnology, Faculty of New Biological Sciences and Technologies, University of Science and Culture, Tehran, Iran
2. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
Email: golian.hosein@gmail.com

Background: Doxepin($C_{19}H_{22}ClNO$) is a serotonin and norepinephrine reuptake inhibitor. Considering this drug's importance in treating neurological diseases, its side effects on the endocrine axes are very important. In this research, the effect of the drug doxepin hydrochloride on the pituitary-gonadal axis and the process of spermatogenesis was investigated.
Materials and Methods: This research was conducted experimentally on 40 adults male Wistar rats in five groups of 8. The control group did not receive any drug treatment. The control group received 2 cc of distilled water daily as a drug solvent. The experimental groups received doses of 40, 80, and 160 mg/kg of doxepin orally for 21 days. Blood was drawn from all groups, on the 22nd day, and the serum concentrations of LH, FSH, and testosterone were measured by radioimmunoassay method. Testicular tissue changes between the experimental and control groups were also investigated. ANOVA and Duncan tests were used for statistical analysis.
Results: The use of doxepin in the amount of 160 mg/kg decreased the testosterone level and increased the concentration of FSH and LH ($P<0.05$). Histological examination of the testicles indicated a clear decrease in the spermatogenic cell chain at the dose of 160 mg/kg.
Conclusion: This study showed that the consumption of doxepin with a concentration of 160 mg/kg significantly decreases the serum concentration of testosterone hormone, weakens the production process of spermatogenic cells, and increases the serum concentration of FSH and LH hormones. Therefore, it is likely that taking this drug with a high dose and duration will reduce the performance of reproductive activity.
Keywords: Doxepin, FSH, LH, Spermatogenesis, Reproduction

P-12: The Protective Effect of Lactobacillus Plantarum on

Cholestasis Induced Testicular Damage based on Histological Parameters

Hajian H[1*], Motallebi M[2], Akhavan Taheri M[3], Kheiripour N[4], Kabiri-Arani S[1], Aghadavod E[1], Shahaboddin ME[4]

1. Department of Clinical Biochemistry, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
2. Department of Immunology and Microbiology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
3. Institute for Basic Sciences, Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran
4. Institute for Basic Sciences, Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
Email: shahaboddin@kaums.ac.ir

Background: The presence of cholestasis and the subsequent buildup of cytotoxic molecules, specifically hydrophobic bile acids, have been linked to various forms of organ damage, including male reproductive system dysfunction, ultimately resulting in male infertility. Various therapies for male infertility have been considered, but probiotics are currently the most highly regarded treatment. There are safety concerns regarding the use of live probiotics in immunocompromised patients. The objective of the present study was to examine the protective effect of heat-killed Lactobacillus plantarum) L. plantarum (against cholestasis-induced histopathological alterations in testicular tissue
Materials and Methods: A total of 32 adult male Wistar rats were evenly divided into four groups of eight rats each: a control normal group, a sham group, and two groups that underwent common bile duct ligation surgery and received distilled water (BDL control) or heat-killed L. plantarum (BDL + L. plantarum) for 28 consecutive days. At the end of the treatment, the rats were sacrificed, and the left testicle was immersed in 10% formalin solution for 24-48 hours to measure histological parameters.
Results: In the BDL+ heat-killed L. plantarum group, the seminiferous tubule diameter, seminiferous tubule area, epithelial height, spermatogenic epithelial area, and spermatogenic epithelial area ratio (%) were significantly higher, while lumen diameter was significantly lower than those in the BDL control group.
Conclusion: Our findings show that heat-killed L. plantarum may be useful for cholestasis-induced histopathological alterations in testicular tissue.
Keywords: Male Infertility, Lactobacillus Plantarum, Cholestasis, Testis

P-13: The Effect of Time-Related Change of Experimental Bile Duct Ligation on Liver Function Tests and Spermatogenesis

Hajian H[1*], Akhavan Taheri M[2], Kabiri-Arani S[1], Ahmadi S[1], Ghanbari M[1], Shahaboddin ME[3]

1. Clinical Biochemistry, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
2. Institute for Basic Sciences, Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran
3. Institute for Basic Sciences, Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
Email: shahaboddin@kaums.ac.ir

Background: Cholestasis is characterized by the severe disruption of bile acid homeostasis. Cholestasis affects the liver more than any other organ. In a bile duct ligation (BDL) animal model of cholestasis, the serum concentration of a wide variety of cytotoxic compounds normally excreted in bile is dramatically elevated. Bile acids are the most suspected renowned compounds implicated in the pathogenesis of cholestasis-associated male reproductive system dysfunction. This study aimed to evaluate the effects of time duration after BDL surgery on the male reproductive system.
Materials and Methods: Twenty-four rats were randomly assigned to sham-operated and BDL groups. Rats in the BDL group were anesthetized, and the common bile duct was localized, double-ligated, and severed between the two ligatures for this purpose. The sham operation involved laparotomy, identification and manipulation of the bile duct without ligation. Each group of animals (Sham-operated and BDL) was euthanized at predetermined time intervals (4, 8, 12, and 16 days after BDL surgery). Blood samples were collected to assess biochemical indicators of liver function, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, and direct bilirubin. In addition, the cauda epididymis of the right testis was separated to examine sperm parameters.
Results: The ALT (4 days after BDL) and ALP (4 and 16 days after BDL) levels were significantly higher than those in the sham group ($P \leq 0.05$). In addition, the sperm quality at 4, 8, 12, and 16 days after BDL was lower than that in the sham group, but this difference was not significant ($P \geq 0.05$).
Conclusion: The results of this study shows that cholestasis has a detrimental effect on liver function and spermatogenesis.
Keywords: Bile Acids, Cholestasis, Male Reproductive System, BDL

P-14: Comparative Analysis of Sperm Parameters and Functional Tests in Fertile Individuals and Men with Varicocele

Hassani M[1*], Nikoozar B[1], Tavalaee M[1], Nasr-Esfahani MH[1, 2]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: mahsa76_h@yahoo.com

Background: Varicocele, characterized by enlarged varicose veins in the scrotum, is the primary identifiable cause of male infertility. Research has shown a strong association between varicocele-related infertility and oxidative stress caused by testicular hyperthermia, leading to poor sperm function. Therefore, we conducted a comparative analysis of sperm parameters and functional tests between fertile individuals and men diagnosed with varicocele.
Materials and Methods: This case-control study utilized a computer-assisted sperm analysis (CASA) system to analyze semen parameters such as sperm concentration, morphology, and motility in 100 fertile men and 100 men with varicocele. To further assess potential factors affecting fertility, oxidative stress, histone residual, protamine deficiency, and DNA damage were also evaluated using bodypi, aniline blue, chromomycin A3, and acridine orange staining, respectively. Statistical analysis was performed using an independent t-test to compare

the study parameters between the two groups, and a p-value of less than 0.05 was considered statistically significant.
Results: The study found that both sperm concentration and motility were significantly lower (P=0.001) in the infertile and varicocele groups compared to the fertile group. On the other hand, the sperm abnormal morphology was higher in the infertile men with varicocele group than the fertile group (P<0.001). Moreover, men with varicocele had significantly higher levels of sperm oxidative stress, histone residual, protamine deficiency, and DNA damage compared to fertile men (P<0.05).
Conclusion: Varicocele is associated with impaired sperm function, including lower sperm parameters, and higher levels of sperm damage due to high oxidative stress caused by heat stress in the testis. The results highlight the importance of early detection and treatment of varicocele to improve male fertility outcomes, including mitigating the effects of heat stress on sperm quality. Further research is needed to better understand the mechanisms involved in varicocele-related oxidative stress and develop more effective interventions for men with varicocele-associated infertility.
Keywords: DNA Damage, Oxidative Stress, Protamine Deficiency, Varicocele, Sperm Parameters

P-15: Apigenin: A Promising Flavonoid for Prostate Cancer Therapy - An In-Depth Investigation Utilizing Network Pharmacology and Molecular Docking

Jabbarzadeh A[1, 2*], Kaveh Vernousfaderani E[1, 2], Bahavar H[1, 2], Farhadi Nejad K[1, 2], Farnam G[3], H. Shirazi F[2, 3]

1. IPharmS, Student Research Committee, Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Pharmacology and Toxicology Department, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: F.shirazi@sbmu.ac.ir

Background: Prostate cancer (PC), with over 1.4 million new cases and 375000 cancer-related deaths in 2020, is the second high incidence diagnosed malignancy in men worldwide, accounting to WHO. As time passes, more studies are found the contribution between male infertility and PC. High expenses, severe side effects, inconsistency and adverse effects on male fertility are the main challenges of currently used treatment options for PC. The utilization of plants and phytochemicals has been a contributory human strategy to treat cancer throughout history. Flavonoids are a group of plants' secondary metabolites consist of over 5000 compounds, found in vegetables and fruits. Studies confirmed various pharmacological effects of flavonoids, such as their antioxidant, anti-inflammatory, and anti-cancer. Apigenin, also known as 4′,5,7-trihydroxyflavone, is a flavonoid found in various food plants and herbs such as onion, oranges, and thyme.
Materials and Methods: In this study, we used Pubchem, Binding DB, SwissTargetPrediction, Similarity Ensemble Approach (SEA), TargetNet, GeneCards, and DisGeNET databases to identify PC-related Apigenin targets. The STRING database was used to explore protein-protein interactions of PC-related apigenin targets. The gene ontology of these proteins calculated by DAVID. Cytoscape 3.9.1 was used to depict the information Network, and Autodock Vina 4.2 performed the molecular docking analysis.
Results: The gene ontology data revealed that signal transduction, cytoplasm, and protein binding are the most probable processes under the influence of apigenin. Based on the network pharmacology, androgen receptor (AR) and sex hormone binding globulin (SHBG) are the most influential targets of apigenin in relation to PC. The affinity of apigenin to AR and SHBG with releasing the energy of -8.4 and -9.3 Kcal/mol, respectively, were the highest affinity of apigenin to PC targets. AR plays an essential role in the progression and growth of prostate cancer. *In vivo* studies have demonstrated that apigenin suppresses the proliferation of androgen-responsive PC cells, which causes a significant drop in the androgen receptor expression. SHBG is an important factor in regulating testosterone levels and can be found abundantly in prostate tissue. Studies have shown that high levels of SHGB can increase the risk of PC.
Conclusion: Considering the results and the most recent study, apigenin appears to be a potential therapeutic agent against prostate cancer due to its ability to affect multiple pathways that stimulate growth and proliferation suppression in prostate cancer cells.
Keywords: Apigenin, Molecular Docking, Network Pharmacology, Prostate Cancer

P-16: Relationships Between Sperm Indices and Semen Total Thiol in Human Subjects with Different Fertility Potential

Javaheri L[1, 2*], Tayefi-Nasrabadi H[2]

1. Tamin Ejtemai Hospital, Shahriar, Iran
2. Department of Basic Sciences, Faculty of Veterinary, Tabriz, Iran
Email: hossein.Tayefinasrabadi@gmail.com

Background: Infertility is considered as one of the most important social and biological-medical problems around the world. Many factors are involved in male infertility, one of which is oxidative stress. Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as thiol containing compounds. The objective of this study was to investigate the relationship between sperm indices and semen total thiol in four groups: normozoospermia, oligozoospermia, asthenozoospermia and oligoasthenozoospermia.
Materials and Methods: According to ethical and scientific protocols, 67 infertile men and 22 normal men were sampled. Sperm parameters (volume, concentration, morphology and motility) were measured in semen samples according to the World Health Organization (WHO) protocol. Semen total thiol was measured spectrophotometrically by using Ellman's reagent or DTNB method.
Results: Results show that the total thiol of semen in patients with pathospermia was significantly lower than the control group (P<0.0001). However, no significant relationship (P<0.05) was found between sperm indices and semen total thiol in normal and infertile men.
Conclusion: In conclusion, a significant reduction in total thiol regeneration in the infertile group compared to the control group indicated the involvement of oxidative stress in male infertility.
Keywords: Oxidative Stress, Sperm Parameters, Total Thiol

P-17: Protective Effects of Ceratonia Siliqua L Extract on Apoptosis and Sperm Chromatin Integrity during The Process of Cryopreservation

Javanmard F[1*], Bidmeshkipour A[1], Ghasemian Yadegari J[2]

1. Department of Biology, Razi University, Kermanshah, Iran
2. Department of Pharmacognosy, Lorestan University of Medical Sciences, Khorramabad, Iran
Email: abidmeshki@razi.ac.ir

Background: Human sperm cryopreservation is a step in the process of fertility treatment that maintains men's fertility for years regardless of their infertility etiology. Sperms are prone to damage during the cryopreservation process due to oxidative stress and apoptotic path induction. Apoptosis is induced during the cryopreservation process, which is followed by the activation of caspases. It must be noted that the antioxidants should be added to the culture medium at the right time and specific dose to prevent negative effects. Some studies have shown the effect of Ceratonia siliqua L extract as an antioxidant on the improvement of sperm chromatin integrity and apoptosis markers after sperm cryopreservation. The present study seeks to investigate the variation in the expression of the genes involved in apoptosis and examine the changes in sperm chromatin integrity in response to the addition of various concentrations of Ceratonia siliqua L extract to the sperm freezing medium in asthenozoospermia specimens
Materials and Methods: Asthenozoospermia specimens were obtained in this study. Each specimen was divided into six groups including groups I (the fresh group) and groups II to VI (with doses of 0 (control), 5, 10, 20, and 30 µgr/ml of Ceratonia siliqua L extract added to the freezing medium). The changes in gene expression and sperm chromatin integrity were then investigated after thawing
Results: Our results indicated that adding the Ceratonia siliqua L extract to the freezing medium maintained sperm chromatin integrity ($P<0.05$). our results also indicated that adding Ceratonia siliqua L extract would reduce the expression of the genes involved in apoptosis (Bax, Caspase-3) and increase Bcl2 gene expression.
Conclusion: Results suggested that the use of various concentrations of Ceratonia siliqua L aqueous-alcoholic extract in the freezing medium could maintain sperm chromatin integrity and reduce the expression of the genes involved in apoptosis in asthenozoospermia cases
Keywords: Asthenozoospermia, Ceratonia, Cryopreservation, Male Infertility

P-18: Effect of Melatonin on Steroidogenesis-Related Enzymes Expression and Testosterone Synthesis Following CoCl2-Induced Hypoxia in TM3 Leydig Cells.

Karimi S[1*], Jalili C[2], Mansouri K[2], Bahremand F[2], Gholami MR[1]

1. Department of Anatomical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: shokoohkarimi7@gmail.com

Background: This study examined the effect of melatonin treatment on steroidogenesis dysfunction and testosterone impairment, following CoCl2-induced hypoxia in TM3 Leydig cells.
Materials and Methods: The TM3 cells were divided into four groups. The first group received no treatment. The MLT group was treated with a concentration of 1 mM melatonin. In the CoCl2 group, 0.2 mM CoCl2 was added to the medium to induce Hif1α (hypoxia-inducible factor 1α) overexpression. The MLT+CoCl2 group received 0.2 mM CoCl2 and 1 mM melatonin. After 24-hour treatment, the cells and supernatants were collected and used for further determination. The MTT assay was performed to estimate the decrease in cell viability throughout the CoCl2 and melatonin treatment. The mRNA and the protein levels were evaluated using Real-time PCR and Western blot analysis. The ELISA assay kit was used to detect the testosterone content.
Results: CoCl2 treatment caused Hif1α overexpression in TM3 Leydig cells. Moreover, CoCl2 treatment of these cells led to considerable downregulation of Star (steroidogenic acute regulatory), Hsd3b1 (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1), and Gata4 (GATA binding protein 4) well as Mtnr1a (melatonin receptor 1a) and Mtnr1b (melatonin receptor 1b) mRNA/protein expression coupled with testosterone content repression in the cell culture medium. Combining melatonin plus CoCl2 treatments decreased Hif1α mRNA/protein expression, but had no significant effect on Star, Hsd3b1, Gata4, Mtnr1a mRNA/protein expression, and the testosterone level in the cell culture medium. Melatonin caused recovery of decrease in the Mtnr1b gene and protein expression.
Conclusion: There was no significant effect on steroidogenesis-related genes, proteins, and testosterone synthesis in the absence of gonadotropin treatment plus melatonin following CoCl2-induced hypoxia in TM3 Leydig cells.
Keywords: Hif1α, Leydig Cells, Melatonin, Testosterone

P-19: The Effect of 6-Dehydrogingerdione and A-Curcumene Zingber Officinale Root in Persian Cuisine on Reproduction and Fertility Outcome

Kheirkhah Rahimabad K[1, 2*], Zavareh Jalal[2]

1. Department of Environmental Health, Sibe Salamat Public Health Institute, Tehran, Iran
2. Department of Culinary Arts, University of Applied Science and Technology, Tehran, Iran
Email: kave.kheirkhah@gmail.com

Background: This study was conducted to effect one of phenolic compound in zingber officinale such as 6-dehydrogingerdione and α-Curcumene as terpene compound in Persian cuisine which prepared with ginger and related between reproduction and fertility outcome, 6 D-G and α-Curcumene the major compounds of zingber officinale root, have received increasing attention due to sexual hormones and semen quality.
Materials and Methods: This study in men consuming Persian foods or supplements containing 40–70 mg/d of 6 D-G and α-curcumene in the previous 3 months was assessed for 30 men ages 35 to 50 years, subjects who presented for semen analyses to our fertility clinics. Linear regression was used to determine the association of Persian cuisine and 6 D-G and α-curcumene with semen quality parameters while adjusting for personal characteristics.
Results: In the multivariate adjusted linear regression models, there was a direct association between 6 D-G and α-Curcumene intake, sperm concentration and total sperm count. Those who have taking higher amounts of ginger, germ cells in about 41 million sperm/ml more than men who did not consume ginger based Persian foods. Relation between zingber officinale food intake and Changes in sperm concentration (Ptrend 0.19), ejaculate volume (Ptrend 0.07), total sperm output (Ptrend 0.11), reach statistical significance.

Conclusion: The intake of ginger-based foods showed many effects on semen quality and sperm concentration. These data support engorges consumption about effects on reproductive hormones and semen quality.
Keywords: A-Curcumene, 6-Dehydrogingerdione, Fertility, Persian Cuisine, Zingber Officinale

P-20: Assessing Sperm Parameters and DNA Integrity: A Comparative Study of Infertile Men with Asthenozoospermia and Normozoospermia

Kiani S[1*], Behdarvandiyan P[1], Nasresfahani A[2], Pashaei K[2], Tavalaee M[1], Nasr-Esfahani MH[1, 2]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, , Isfahan, Iran
Email: shaghayegh.kiani99@gmail.com

Background: Asthenozoospermia is a condition characterized by reduced or lack of sperm motility in male ejaculation. Several studies have shown a correlation between sperm motility and DNA integrity. As DNA integrity is essential for the success of infertility treatment and the health of the next generation, we aimed to assess sperm DNA damage using SCSA and TUNEL tests in a large population of 500 asthenozoospermic men and 500 normozoospermic men. This study is the first to assess sperm DNA damage in a large population of men with asthenozoospermia.
Materials and Methods: In this comparative study, sperm concentration, motility, abnormal morphology, and semen volume were evaluated in accordance with the 2010 World Health Organization guidelines. To compare the study variations between the asthenozoospermic and normozoospermic groups, we used an independent sample t-test. In addition, for the correlation between the study parameters, Pearson's correlation coefficient was used. Statistical significance was set at $P < 0.05$.
Results: Unlike semen volume and male age, which were similar between the two groups, the mean sperm concentration and total motility were significantly lower in asthenozoospermic men than in normozoospermic men ($P<0.001$). In addition, the mean value of sperm DNA fragmentation assessed using SCSA and TUNEL assays was significantly higher in asthenozoospermic men than in normozoospermic men ($P<0.001$).
Conclusion: In asthenozoospermic individuals, the level of sperm DNA damage was significantly high. Antioxidants have been shown to have a protective effect against sperm DNA damage. Therefore, treating asthenozoospermic men with antioxidant therapy may improve their sperm quality and increase the level of pregnancy. Additionally, novel sperm selection procedures can be used to select sperm with better DNA integrity for use in assisted reproductive techniques.
Keywords: Sperm Parameters, DNA Fragmentation, Sperm Motility

P-21: Association between Varicocele and Testosterone Levels: A Prospective Cohort Study

Lotfalian A[1*], Anvari M[1], Khaledi S[2]

1. Biology and Anatomical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: azam.lotfalian@yahoo.com

Background: Varicocele, a common condition characterized by the enlargement of veins in the scrotum, has been associated with hormonal imbalances, including reduced testosterone levels. However, the exact relationship between varicocele and testosterone levels remains unclear. This prospective cohort study aimed to investigate the association between varicocele and testosterone levels in a cohort of patients.
Materials and Methods: n total, 200 male patients with clinical varicocele were enrolled in the trial and monitored for a year. Physical examination, scrotal ultrasonography, semen analysis, and the testing of blood testosterone levels were all part of the baseline evaluations. Clinical examinations and measurements of blood testosterone levels were part of follow-up evaluations that were carried out six and twelve months after the baseline.
Results: With a mean blood testosterone level of 225 ng/dL at baseline, 77% of the patients had abnormal testosterone levels, which are defined as serum testosterone levels below 280 ng/dL. With a mean serum testosterone level of 344 ng/dL at 6 months after baseline, 61% of the patients had improved serum testosterone levels. With a mean serum testosterone level of 373 ng/dL at 12 months after baseline, 71% of the patients had improved serum testosterone levels. It was statistically significant that the serum testosterone levels had increased ($p = 0.001$). When compared to patients with lower-degree varicocele (grade II), those with higher-grade varicocele (grade III) displayed a greater recovery in serum testosterone levels. Patients who underwent surgical therapy for varicocele and those who received conservative management experienced similar improvements in their testosterone levels.
Conclusion: Significant numbers of people with varicocele also have lower serum testosterone levels. However, varicocele treatment, whether surgical or nonsurgical, is linked to a gradual rise in serum testosterone levels. For patients with varicocele and hormone abnormalities, additional research is necessary to understand the underlying mechanisms of this connection and to choose the best course of treatment.
Keywords: Infertility, Testosterone, Varicocele

P-22: Follow-up of Varicocele Embolization: Effectiveness and Safety in A Cohort Study

Lotfalian A[1*], Anvari M[1], Khaledi S[3]

1. Department of Biology and Anatomical Sciences, Shahid Sadoughi University of Medical sciences, Yazd, Iran
2. Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: azam.lotfalian@yahoo.com

Background: Varicocele is a common condition that can cause male infertility and testicular discomfort. Varicocele embolization, a minimally invasive procedure, has gained popularity as an alternative to surgical treatment for varicocele. However, there is limited data on the long-term effectiveness and safety of varicocele embolization. This original article presents the results of a large cohort study examining the long-term outcomes of varicocele embolization.
Materials and Methods: The study included 500 clinical varicocele patients who had varicocele embolization at a single center. Physical examination, scrotal ultrasonography, semen analysis, and a visual analog scale (VAS) to measure testicular discomfort were all part of the baseline evaluations. Following

embolization, patients were followed up to measure semen parameters, testicular discomfort, and complications at 6 months, 1 year, and 2 years.
Results: Varicocele embolization had an overall success rate of 95% at 6 months, 94% at 1 year, and 88% at 2 years after the procedure, which was determined by the absence of varicocele on clinical examination and scrotal ultrasonography. The success rates were comparable across grade II and grade III varicoceles as well as between coil embolization and sclerotherapy as embolization procedures. Overall, only 7% of patients experienced moderate issues, whereas 2% of patients experienced serious difficulties, both of which were successfully treated.
Conclusion: With continuous improvements in semen parameters and testicular discomfort during long-term follow-up, varicocele embolization is a safe and effective therapeutic option for varicocele. When it comes to treating varicocele, varicocele embolization may be a good alternative to surgery, especially for people who don't want to have surgery or who have medical reasons not to.
Keywords: Embolization, Male Infertility, Varicocele

P-23: A Prospective Randomized Controlled Trial Comparing Microscopic Subinguinal Varicocelectomy and Laparoscopic Varicocelectomy: A Single-Center Experience

Lotfalian A[1*], Anvari M[1], Khaledi S[2]

1. Department of Biology and anatomical sciences, Shahid Sadoughi university of medical sciences, Yazd, Iran
2. Department of Anatomy, Faculty of medical sciences, Tarbiat Modares University, Tehran, Iran
Email: azam.lotfalian@yahoo.com

Background: Male infertility due to varicocele is a prevalent problem, and patients with symptomatic varicocele or decreased fertility are frequently advised to undergo surgery. Microscopic subinguinal varicocelectomy and laparoscopic varicocelectomy are two commonly used surgical techniques, but their comparative efficacy and safety remain unclear. This prospective randomized controlled trial aimed to compare the outcomes of microscopic subinguinal varicocelectomy and laparoscopic varicocelectomy in patients with varicocele.
Materials and Methods: Two groups of 120 patients with clinical varicocele were randomly assigned: Group A received microscopic subinguinal varicocelectomy, whereas Group B got laparoscopic varicocelectomy. Physical examination, scrotal ultrasonography, and semen analysis were all part of the preoperative evaluation. At 3, 6, and 12 months after surgery, the results of the operation were evaluated. These outcomes included success rates (defined as absence of varicocele on clinical examination and scrotal ultrasonography), recurrence rates, postoperative discomfort, comorbidities, and changes in semen parameters.
Results: There were no statistically significant differences between the two groups, and both demonstrated considerable postoperative improvements in semen parameters. At 12 months postoperatively, the success rates were comparable in Groups A (84%) and B (83%) with no appreciable difference in the recurrence rates (5% in Group A, 6% in Group B). At 3- and 6-months following surgery, Group A experienced much less postoperative discomfort than Group B; however, there was no discernible difference at 12 months following surgery. Both groups experienced minimal rates of problems, and no significant issues were found.
Conclusion: With comparable success and recurrence rates, laparoscopic and microscopic subinguinal varicocelectomy are both effective surgical procedures for the treatment of varicocele. In the immediate postoperative period, microscopic subinguinal varicocelectomy may be associated with less postoperative pain than laparoscopic varicocelectomy, but the difference gradually disappears.
Keywords: Laparoscopic, Subinguinal, Varicocelectomy

P-24: Silymarin Therapy Inhibitory Role on Testicular Torsion/Detorsion-Induced Damages in Rats

Madani J[1*], Asgari Navidian M[1], Seyyed Anvari S[2], Gharibeh N[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Department of Biology, Collage of Post Graduate, Ahar Islamic Azad University, Ahar, Iran
Email: Samira.s.anvari@gmail.com

Background: Various antioxidants have been studied for their potential in mitigating testicular torsion/reperfusion (TT/TR) injuries. Silymarin (SMN) is a well-known antioxidant and anti-inflammatory agent that has shown promise in improving infertility. This study aimed to evaluate the effects of SMN on TT/TD.
Materials and Methods: Thirty-two male mature Wistar rats were randomly divided into sham and experimental groups (n=8 rats/group). TT was induced in twenty-four rats for one hour, with eight rats assigned to the TT group. Additionally, sixteen rats underwent testicular detorsion (TD) for one hour. Among them, eight animals received normal saline (TD/NS group), while the remaining eight received SMN (200mg/kg; TD/SMN200 group), injected 30 minutes before TD surgery. Testicles were dissected for histopathological (TDI, SPI, and RI) and immunohistochemical (p53, Caspase3) analysis.
Results: The number of p53 and Caspase-3 positive cells per mm2 of tissue were higher in the TT and TD/NS groups compared to the sham group. Furthermore, spermatogenesis was impaired, and the percentages of positive TDI, SPI, and RI were reduced in the TT and TD/NS groups compared to the sham group. Treatment with SMN enhanced the TDI, SPI, and RI percentages while reducing the number of p53 and Caspase-3 positive cells in the TD/SMN200 group compared to the TT and TD/NS groups.
Conclusion: Based on the findings of this study, it can be concluded that a single injection of SMN (200mg/kg) administered 30 minutes before TD surgery positively improved germ cell survival rate in the testis, leading to an increase in TDI, SPI, and RI. These results suggest that SMN holds potential as a protective agent against TT/TR injuries.
Keywords: Silymarin, Testicular Detorsion, Testicular Torsion, Rat

P-25: Co-supplementation of Trehalose and Lecithin Preserved Human Sperm Quality following Vitrification

Marofi MS[1*], Rezaei Topraggaleh T[1, 2], Pourheydar B[1], Farjah G[1], Bolbole S[1], Karimipour M[1]

1. Department of Anatomical Sciences, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2. Reproductive Health Research Center, Clinical Research Institute, Urmia University of Medical Sciences, Urmia, Iran

Email: mojtaba_karimipour@yahoo.com

Background: Sperm cryopreservation is considered as a valuable therapeutic option in male fertility management. Vitrification or ultra-rapid freezing is rarely used for cryopreservation of human spermatozoa due to low volume of sperm cytoplasm. However, this method is simple, user friendly which does not require an expensive equipment and cryoprotectants. Human serum albumin is widely used for vitrification of human spermatozoa. However, batch to batch variation and the possibility of contamination with microbial agents are major drawback of serum albumin. Therefore in this study we aimed to substitute human serum albumin with co-supplementation of trehalose and lecithin in vitrification solution of human spermatozoa.
Materials and Methods: Twenty semen sample were collected form normozoospermic men based on WHO guidelines. After swim-up, samples were divided to five equal group, control and four treatments groups containing trehalose 0.25 M and 0.5 M and lecithin 0.5 and 1 % as following T25L05, T25L10, T50L05 and T50L10. Sperm samples were frozen by vitrificcation protocol and sperm motility, viability, membrane integrity, mitochondrial membrane potential and DNA fragmentation index were analyzed after warming.
Results: The T025L10 group showed the highest post warming motility, progressive motility, viability, membrane integrity and mitochondrial potential. However, supplementation of higher concentration of trehalose decrease sperm quality compared to control group ($P<0.05$). Percentage of spermatozoa with large and medium halo were significantly higher in T25L05 and T25L10 groups compared to control group. While there were no statistically significant differences among the T50L05 and T50L10 and control group in DNA integrity.
Conclusion: Our result showed that, co-supplementation of trehalose and lecithin (T025L10) in vitrificcation solution by increasing motility, viability, membrane integrity and mitochondrial potential as well as decreasing DNA fragmentation, improved post-warming quality of human spermatozoa.
Keywords: Lecithin, Sperm, Trehalose, Vitrification

P-26: Exploring Sperm DNA Fragmentation in Severe Asthenozoospermic Men

Naderi N[1*], Tavalaee M[1], Nasr-Esfahani A[1, 2], Pashaei K[2], Nasr-Esfahani MH[2]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
Email: nushin.naderi1987@gmail.com

Background: Successful fertilization is dependent on sperm motility, which allows them to penetrate the oocyte. When less than 42% of sperm exhibit motility, it is called asthenozoospermia, a common cause of male infertility. The aim of this study is to investigate the correlation between sperm DNA damage, sperm parameters, age, and body mass index (BMI) in cases of severe asthenozoospermia (< 2% sperm motility) and normozoospermia.
Materials and Methods: The study analyzed 50 subjects with severe asthenozoospermia and 50 subjects with normozoospermia. Sperm parameters were evaluated according to WHO 2010 guidelines, and sperm DNA damage was measured with TUNEL and SCSA methods. The non-parametric Mann-Whitney U tests were utilized to compare the parameters between the two groups, and Pearson's correlation coefficient was used to assess the relationship between sperm DNA damage, sperm parameters, age, and BMI. The significance level was set at $P < 0.05$.
Results: The study showed that severe asthenospermic individuals had lower mean sperm concentration and count, as well as lower percentages of sperm total motility and progressive motility ($P < 0.001$) compared to normozoospermic individuals. Additionally, severe asthenozoospermic individuals had a significantly higher mean percentage of sperm DNA damage ($P < 0.001$), and there was a positive correlation between sperm DNA damage and the age of severe asthenozoospermic men.
Conclusion: Sperm motility and DNA damage, and paternal age affect embryo development. Assessing DNA damage is crucial in evaluating male fertility and selecting treatment for asthenozoospermia. This helps identify causes of infertility and develop personalized treatment plans for successful conception and healthy embryo development.
Keywords: Asthenozoospermia, Male Infertility, SCSA, Sperm DNA Damage, TUNEL

P-27: The Effect of Listeria Monocytogenes-Induced Meningitis on Sperm Parameters Testes in C57 Male Mice Model

Pouriayevali F[1, 2*], Karimi-Dehkordi M[2]

1. Department of Animal Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
Email: ma_karimivet58@yahoo.com

Background: Listeria monocytogenes, the chief species of the Listeria genus, is a Gram-positive intracellular bacterium distributed in the environment, which can grow at 4°C. It is generally transmitted to humans through ingestion of contaminated food. Listeria monocytogenes is a common source of bacterial meningitis. Therefore, this study aimed to find an association between bacterial meningitis and blood factors with sperm parameters.
Materials and Methods: In this research, twenty male C57 male mice (6-8 weeks) were randomly divided into control groups and Listeria monocytogenes-induced meningitis group (LM) received 108 CFU/ml intracisternal injections. After 10 days, all of the mice were sacrificed and sperm parameters including sperm motility, sperm concentration, and sperm morphology were measured. In addition, blood samples were collected from the heart, and were gathered for valuation of white blood cells, neutrophils, eosinophils, monocytes, lymphocytes, basophil, total antioxidant capacitation (TAC), and glutathione peroxidase (GPx).
Results: In this research indicated that bacterial meningitis could increase white blood cells and neutrophils ($P=0.008$) in the LM group but other blood factors including eosinophils, monocytes, lymphocytes, basophils hadn't a significant difference between the control group and the LM group. In addition, the blood level of TAC and GPx were higher in the LM group compared to the control group ($P=0.000$). The mean percentage of immotile sperm, sperm concentration, and sperm abnormal morphology were higher in the LM group than the control group ($P=0.003$).
Conclusion: According to the findings of this study, Listeria

monocytogenes-induced meningitis caused increasing inflammatory factors in the blood that caused to injure the sperm cells. Thus meningitis can affect the sperm cells.
Keywords: Listeria Monocytogenes, Meningitis, Sperm Parameters

P-28: *In Vitro* Effect of Fluoxetine on Human Sperm Parameter and DNA Integrity

Roostaee Z[1*], Soleimani Mehranjani M[1], Cheraghi E[2], Shariatzadeh Seyed MA[1]

1. Department of Biology, Arak University, Arak, Iran
2. Department of Biology, Qom University, Qom, Iran
Email: roostaeez143@gmail.com

Background: Fluoxetine, has the highest prescription rate in treating depressive disorders due to its effectiveness and safety. However, this drug can cause oxidative stress and hence affect cell viability. Considering that many men who are at the reproductive age often use this drug for a long period of time, it is necessary to investigate its effect on sperm cells *in vitro*.
Materials and Methods: Semen samples of 30 fertile men were collected. Each sample was divided into three groups: fresh, control (without treatment and 1 hour incubation) and fluoxetine (treated with 5 μM fluoxetine and 1 hour incubation). Then the samples were evaluated in terms of sperm parameters (progressive, non-progressive motility, total motility, morphology and viability) and sperm DNA fragmentation and reactive oxygen species (ROS). Data were statistically analyzed using repeated measures analysis.
Results: The total and progressive motility, viability and normal morphology of sperm showed a significant decrease in the fluoxetine group compared to the fresh and control groups ($P<0.001$). while, a significant increase in the mean percentage of nonprogressive motility, DNA fragmentation and ROS was observed in the fluoxetine group compared to the fresh and control groups ($P<0.001$).
Conclusion: Our study showed that fluoxetine, leads to a decrease in total and progressive motility, viability, normal morphology and an increase in nonprogressive motility and sperm DNA fragmentation through increasing ROS.
Keywords: Fluoxetine, Depression, Human Sperm, Oxidative Stress

P-29: The Effect of N-Acetylcysteine on Motility, Acrosome Reaction and DNA Integrity of Human Sperm: An *In Vitro* Study

Roostaee Z[1*], Soleimani Mehranjani M[1], Cheraghi E[2], Shariatzadeh Seyed MA[1],

1. Department of Biology, Arak University, Arak, Iran
2. Department of Biology, Qom University, Qom, Iran
Email: roostaeez143@gmail.com

Background: N-Acetylcysteine (NAC) is a thiol-containing compound with strong antioxidant properties that can be effective in the treatment of infertility, cardiovascular diseases, human immunodeficiency virus infections, liver poisoning and metal poisoning. Since the effect of NAC on normal sperm cells has not been measured and it is necessary to understand the mechanism of its effect on sperm cells before considering its use in clinical conditions, our aim is to investigate the effect of NAC on motility, acrosome reaction and DNA integrity of normal sperm.
Materials and Methods: Normal sperm samples of 30 fertile men were collected. Then, each sample was divided into three groups: fresh, control (untreated after 1 hour of incubation) and NAC (treated with 50 μM NAC antioxidant after 1 hour of incubation). The sperm mobility in each sample was measured with a light microscope. Also, acrosome reaction and DNA fragmentation were evaluated using FITC- Pisum sativum agglutinin and acridine orange staining, respectively. The data was analyzed using repeated measures analysis.
Results: A significant increase in the mean sperm total and progressive motility was observed in the NAC group compared to the fresh and control groups ($P<0.001$). NAC was able to significantly reduce the nonprogressive motility, premature acrosomal reaction and sperm DNA fragmentation compared to the control group ($P<0.01$).
Conclusion: Our observations showed that NAC has positive effects on normal sperm and can improve progressive and total sperm motility, DNA integrity and acrosomal integrity.
Keywords: DNA Integrity, N-acetylcysteine, Normal Sperm

P-30: The Protective Effect of Silymarin on Serum Biochemical Factors in Adult Mice after Treatment with Cyclophosphamide

Shaker Z*, Soleimani mehranjani M, Ahmadi S

Department of Biology, Arak University, Arak, Iran
Email: zahrashakker@gmail.com

Background: Cyclophosphamide is an alkylating anticancer agent with high efficacy; however, it also causes oxidative stress, which can lead to biochemical disorders. Antioxidants play a very important role in dealing with oxidative damage. We aimed to investigate the effect of silymarin as an antioxidant on testis function in adult male mice after treatment with cyclophosphamide.
Materials and Methods: In this study, 24 NMRI male mice (37±2 g) were divided into 4 groups (n=6): control; cyclophosphamide (100 mg/kg bw/week, ip); Silymarin (200 mg/kg bw/interval day, ip) and cyclophosphamide + silymarin. After 35 days of treatment, the serum samples were collected to measure the testosterone level, total antioxidant capacity (TAC) and malondialdehyde (MDA) level. The obtained data were statistically analyzed using one-way ANOVA and Tukey's test and the means were considered significantly different at $P<0.05$.
Results: A significant decrease in the level of testosterone hormone and the total antioxidant capacity (TAC) was observed in the cyclophosphamide group compared to the control group, while the level of malondialdehyde (MDA) significantly increased in the cyclophosphamide group compared to the control group. In the cyclophosphamide + silymarin group, compared to the cyclophosphamide group, there was a significant decrease in the amount of MDA ($P<0.01$), a significant increase in the TAC ($P<0.05$) and an increase in the testosterone hormone level. The mentioned parameters did not show any significant difference in the silymarin group when compared to the control group ($p>0.05$).
Conclusion: Our results indicated that silymarin, as a strong antioxidant, can prevent the adverse effects of cyclophosphamide and improve the function of mice testis through reducing oxidative stress and increasing total antioxidant capacity.
Keywords: Cyclophosphamide, Mice, Oxidative Stress, Silyma-

rin, Testosterone

P-31: Correlation between Serum Homocysteine Levels with Sperm Chromatin, and Oxidative Stress in A Rat Model of Varicocele"?

Shiasiy[1, 2*], Monajemi R[1], Tavalaee M[2], Nasr-Esfahani MH[2]

1. Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: shshshazam@gmail.com

Background: High homocysteine levels have been linked to vascular disease-related oxidative stress. Varicocele, a dilation of the spermatic vein, is the leading cause of male infertility due to oxidative stress resulting from heat stress or vasodilator production. We aimed to study the impact of varicocele induction on rat plasma homocysteine levels, sperm function, and parameters.
Materials and Methods: We divided thirty Wistar rats into three groups (varicocele, sham, and control) and analyzed them two months after varicocele induction. We evaluated their sperm parameters, oxidative stress (using Bodipy staining), and DNA damage (using acridine orange). To compare variations between groups, we used one-way ANOVA and considered a p-value less than 0.05 to be significant.
Results: The varicocele-induced group showed a significant decrease in sperm parameters, including count, motility, and morphology, compared to the control and sham groups ($P<0.001$). Moreover, plasma homocysteine level, oxidative stress, and sperm DNA damage were also significantly reduced in the varicocele-induced rats compared to the control and sham groups ($P<0.05$).
Conclusion: Our study showed a significant decrease in sperm parameters and an improvement in plasma homocysteine level, oxidative stress, and sperm DNA damage in the varicocele-induced rats compared to the control and sham groups. These findings suggest a potential therapeutic approach for male infertility associated with varicocele through the management of oxidative stress.
Keywords: Homocysteine, Oxidative Stress, Sperm DNA Damage, Sperm Parameters, Varicocele

P-32: Reduction of The Negative Effects of Heat Stress on Male Infertility Treated by Bioactive Peptide Derived from Sardinestle

Valizadeh M[1, 3*], Nejati V[1], Shalizar Jalali A[2], H. Najdgerami E[1], Najafi G[2], Delkhosh-Kasmaie F[3]

1. Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR , Tehran, Iran
Email: mahsa_valizadeh20@yahoo.com

Background: Heat is a damaging environmental factor that disrupts spermatogenesis and results in male infertility. investigations have shown that heat stress reduces the fertilization ability of living spermatozoa. with their antioxidant effects, Bioactive peptides play an important role in the metabolic function of organisms and human health.
Materials and Methods: In the present study, 56 adults male Wistar rats were randomly categorized into 8 groups (n=7) including group 1: Control, groups 2: Bioactive peptides (10 mg/kg/day; PO), groups 3, 4, and 5: Heat-stressed (37, 39 and 43°C for 20 min per day, respectively) and groups 6, 7 and 8: Heat-stressed along with bioactive peptides (37, 39 and 43°C for 20 min per day respectively plus bioactive peptides at a dose of 10 mg/kg/day; PO). The heat stress was induced through the immersion of rat scrotums in a water bath. After 45 days, rats were sacrificed and left testes were removed, fixed, and used for histological and immunohistochemical studies. Harvested right testes were also used for oxidative stress assessments and molecular analyses.
Results: Heat stress increased testicular tissue damage, elevated oxidative stress and reactive oxygen species production and increased germ cell apoptosis, P53 and Caspase 3 expressions and Bax/Bcl-2 ratio ($P<0.05$). Treatment with bioactive peptide as a substance with antioxidant properties ameliorated the damage caused by heat stress($P<0.05$).
Conclusion: The results of this study highlight the protective role of bioactive peptides in the reproductive tract under heat stress and their potential function against oxidative stress and apoptosis in testicular tissue.
Keywords: Bioactive Peptide, Oxidative stress, Heat Stress, Rat, Testis

P-33: Ability and Accuracy of The Smartphone-Based SPOO® Sperm Test Based on Machine Vision

Vaseghi Dodaran H[1*], Atshan M[2], Pousti G[3]

1. Department of Animal Science, University of Tabriz, Tabriz, Iran
2. Department of Biology, Faculty of Science, Arak University, Arak, Iran
3. Department of Biomedical Engineering, Old Dominion University, Norfolk, VA 23529, USA,
Email: h.vaseghi28@yahoo.com

Background: Unfortunately, one in six couples is affected by infertility in the world. It is interesting to know that men and women have an equal share of this problem. Diagnosis is the first step in solving this problem. Usually, couples go to the laboratory to take a test but this process can be very embarrassing, expensive, time-consuming, and stressful. Among couples presenting for infertility assessment, 18 to 27% of men will not be tested. We designed a self-test medical device (SPOO) enabling men to test, analyze, and improve their sperm quality from the comfort of their own homes. SPOO is a smartphone-based home sperm test application that evaluates sperm count, motility, and morphology and gives a complete fertility report that can be shared with a doctor. The aim of this paper is to introduce a smartphone-based Home Sperm Test accurately and precisely measure sperm total motility, morphology and count versus the CASA, an automated laboratory semen analyzer.
Materials and Methods: 350 human semen samples were tested by professionals at two sites utilizing the SPOO Home Sperm test kit. In parallel, the same samples were tested on the CASA automated semen analyzer (VIDEO TEST SPERM 3.1). Samples were collected, liquefied, split and run in a blinded fashion. Professionals ran the SPOO test using the SPOO device on either a Galaxy Smartphone following the SPOO app.

SPOO uses the smartphone's camera and light source and the SPOO Clip (a mini-microscope) to capture a moving sperm video. Using proprietary algorithms, the app analyzes the video and translates these movements into sperm count, motility and morphology.
Results: The SPOO device demonstrated good correlation and good to moderate agreement with the CASA for count and total motility parameters.
Conclusion: The smartphone based device (SPOO) is affordable and convenient option for men wanting an answer about their fertility as soon as possible. The smartphone-based device has a high level of accuracy and precision when compared with the CASA. In the absence of a clear-cut evaluation and diagnosis of the male partner, the female partner may undergo unnecessary and unsuccessful medical interventions. This highlights the need for an at-home semen screening test that is relevant, accurate, easy to use, and affordable. Thus, the SPOO can improve patient satisfaction and empowerment.
Keywords: Artificial Intelligence, Infertility Sperm

Animal Biotechnology

P-34: Protective Effect of Alginate Against Vitrification Damage in Mouse Ovarian Tissue

Aminian S[1*], Akhavan Taheri M[2]

1. Department of Gametogenesis Research, Center, Kashan University of Medical Sciences, Kashan, Iran
2. Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran
Email: maryam.akhavantaheri@yahoo.com

Background: Cryopreservation of ovarian tissue is considered a useful method for fertility preservation. This study aimed to preserve most of the follicular reserve from the destructive effects of cryoprotectant solutions and liquid nitrogen.
Materials and Methods: In this empirical study, ovaries from female NMRI mice (8 weeks old) were randomly divided into four groups: Fresh (not vitrified), Vitrification (not encapsulated vitrified), Alginate 1 (encapsulated in 1% alginate hydrogel before vitrification protocol), Alginate 2 (encapsulated in 1% alginate hydrogel before placing in liquid nitrogen) After vitrification and warming, histological structure, gene expression (Bax, Bcl2, P53, Kit), and oxidative stress levels (NO test and MDA test) were examined in each group.
Results: Histological evaluation showed that the highest number of intact follicles was found in the Fresh group and the lowest in the Aloe vera 1 group. The average number of primordial follicles in the Alginate 2 group increased compared with the Vitrification group, although this increase was not statistically significant, it showed a significant decrease compared with the Fresh group ($P<0.05$). Results of evaluating the expression of apoptosis-related genes showed that the ratio of Bax/Bcl2 and P53 significantly decreased in the Alginate 2 group compared with the vitrification group. The level of Kit gene expression was either the same or lower in the experimental groups than in the vitrification group, but there was no statistically significant difference. Levels of tissue nitrate, nitrite, and malondialdehyde in Alginate groups 1 and 2 showed a significant decrease compared with the vitrification group ($P<0.05$).
Conclusion: : Encapsulation of ovaries in 1% alginate hydrogel before immersion in liquid nitrogen may reduce the damage caused by cryopreservation.
Keywords: Alginate, Aloevera, Mouse, Ovary, Vitrification

P-35: Cell Culture and Cryopreservation of Sexual Tissues of Three Iranian leopard Cheetahs

Ashouri Movassagh S*, Farghadan M

Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
Email: ashouri_sepideh@yahoo.com

Background: The conservation of the wildlife animals, as the valuable genetic reserves, is one of the important policies of the countries. This issue becomes more important especially in relation to endangered species. The Iranian cheetah is one of the species that is seriously endangered. In this study, in order to preserve the genetic and biological resources of the country, we extracted the cells from the skin and testicle tissue, as well as cryopreservation the ovary and testicle tissue of 3 Iranian leopard Cheetahs.
Materials and Methods: Immediately after the death of Iranian cheetah, skin tissue and sexual tissue samples were taken from the animal. The samples were transferred to the laboratory in the shortest time in the culture medium containing antibiotics at 4°C. In the laboratory, skin tissue and some testicular tissues were cultured by explant culture and enzymatic digestion, and some testicular and ovarian tissues were cryopreserved by slow method.
Results: The cells extracted from skin tissue and testicle tissue were frozen after 4 weeks of culture after quality control tests. The frozen ovary and testis tissues were stored in a nitrogen tank for long-term storage.
Conclusion: Cryopreservation the cells and sex tissue of endangered animals such as the Iranian leopard cheetah is a way to preserve their genomic information and hope for the revival of this endangered species.
Keywords: Conservation, Cryopreservation, Endangered Species, Sexual Tissues,

P-36: Changes in lactic Acid Bacteria and Total Bacteria of Rumen of Gray Shirazi Estrus and Anestrus Ewes in The Estrous Cycle of The Non-Breeding Season

Bolooki Z*, Jafarzadeh Shirazi MR

Department of Animal Science, Faculty of Agriculture, Shiraz University, Shiraz, Iran
Email: jafarzd@shirazu.ac.ir

Background: In this study, the main goal is to investigate the changes in the rumen microbial population during the non-breeding season in the estrous period for estrus and anestrous ewes.
Materials and Methods: To investigate the changes in rumen fluid microbial population during the estrous cycle of estrus and anestrus animals, two groups of 10 estrus anestrus animals were formed, and rumen fluid samples were taken from every four days for microbial culture and other investigations in both groups.
Results: The population difference between the two groups at 5 different times showed that only on the day of estrus, the population of lactic acid producing bacteria has a higher value and a significant difference compared to the anestrous group. The comparison of bacteria population of two estrus and anestrus

groups showed that the 13th and 17th days of estrus have higher values and a significant difference compared to other times. The total bacterial population was compared with the estrus and anestrus groups, this comparison showed that the amount of the total bacterial population in the estrus group was higher and significant at all times.
Conclusion: Overall, in the study, the cultures obtained from the rumen fluid in the estrus group showed that the total colony population of anaerobic bacteria has a significant difference on certain days with the anestrus group.
Keywords: Estrus, Ewe, Reproductive, Rumen, Season

P-37: Protective Effects of Coenzyme Q10 on Sperm Parameters, in Mouse Model of Hemolytic Anemia

Dibayi Asl Z[1*], Karimipour M[1], Ahmady A[2]

1. Department of Anatomical Sciences, Faculty of Medicine, Urmia university of Medical Sciences, Urumia, East Azarbayjan, Iran
2. Department of Basic sciences, Faculty of Veterinary, Urmia University, Urmia, East Azarbayjan, Iran
Email: zahra.dibayi.213@gmail.com

Background: Hemolytic anemia is a condition in which the lifespan of red blood cells decreases, resulting in hypoxia and oxidative stress. These factors can damage the testis structure, cause sperm dysfunction, and eventually lead to male infertility. The objective of this study was to evaluate the protective effect of coenzyme Q10 (CoQ10) on sperm in a mouse model of hemolytic anemia induced by phenylhydrazine (PHZ).
Materials and Methods: Thirty-two NMRI adult male mice (n = 8/each) were randomly classified into 4 groups: the control group (tween 20 1%, 0.1 ml/day IP), the PHZ group (8 mg/100g IP at the first week, followed by 6 mg/100g every 48 hours), the CoQ10 group (10 mg/kg, daily IP), and the PHZ+CoQ10 group. After 35 days, sperm parameters (count, motility, viability, and normal morphology) were evaluated. Also, sperm chromatin structure assay (SCSA) and sperm chromatin maturity assay (SCMA) were performed on sperm samples.
Results: A significant ($P<0.001$) reduction in sperm count, motility, viability, and normal morphology was observed in the PHZ group compared with the control group. In the PHZ+CoQ10 group, these parameters improved significantly ($P<0.05$) compared with PHZ-treated mice. The percentage of sperm with DNA damage and sperm with immature nuclei significantly increased ($P<0.001$) in the PHZ group versus the control group. In anemic mice that received CoQ10, the percentage of sperm with DNA damage ($P<0.001$) and the percentage of sperm with an immature nucleus ($P<0.05$) significantly decreased compared with the PHZ group.
Conclusion: The findings of this study indicate that hemolytic anemia causes a severe decrease in sperm quantity and quality. CoQ10 refines defects with its antioxidant and antiapoptotic activities, but cannot completely cure the damages.
Keywords: Coenzyme Q10, Hemolytic Anemia, Phenylhydrazine, Sperm Parameters

P-38: Probing The Effect of Aloevera Plant Extract on Reproductive Hormones in Asthmatic Female Rats Treated with Acetic Acid

Gholizadeh N[*], Farrokhi F

Department of Biology, Urmia university, Urmia, Iran
Email: nahidgholizade69@gmail.com

Background: In the Liliaceae family, aloe vera is a perennial plant. Studies have shown the presence of antibacterial, anticancer and anti-inflammatory characteristics on leaves from this plant. This research showed that extracts from the aloe vera plant are capable of influencing animal reproductive physiology. In addition, it appears that extracts fromAloe Vera may have an impact on animal reproductive physiology. The research also indicates that aloe Vera plant extracts can impact animals' reproductive physiology.
Materials and Methods: In three groups of 6 rats each, 18 mature female Vista strain rats weighing approximately 160+20 kg were used in this experimental study. No substances were received in the 1st group. In the second and third groups, asthma was induced by 0.1 mg of acetic acid as a spray for 2 hours daily for 8 weeks.. The first group received no substance. In the second and third groups, asthma was induced by 0.1 mg of acetic acid as a spray for 2 hours daily for 8 weeks. A third group of asthmatic patients received 100 mg of aloe vera extract orally for eight weeks. Finally, the rats were anesthetized with ether and the ventricles were bled, by which serum hormones were.
Results: Studies have shown that estrogen and progesterone levels were significantly reduced in the asthmatic group. And, in the third experimental group that took the extract, the amount of progesterone did not witness any significant changes, but the amount of estrogen rose markedly.
Conclusion: Results illustrate that aloe vera extract includes phytoestrogenic compounds like sitosterol, which have estrogenic properties and may increase levels of estrogenic hormones.
Keywords: Aloevera, Asthma, Estrogen, Rat, Reproductive hormones

P-39: The Effect of Perpolis Coated with Chitosan Nanoparticles on The Insulin Resistance Index and Serum Factors of The Polycystic Ovarian Syndrome Animal Model

Hosseini F[1*], Khodaei F[2]

1. Department of Anatomy, Birjand University of Medical Sciences, Birjand , Iran
2. College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, China
Email: seyedefatemeh.hosseini@bums.ac.ir

Background: Polycystic ovary syndrome (PCOS) is a type of hormonal disorder that associated with metabolic dysfunction. Studies have shown that type 2 diabetes mellitus and PCOS are related due to insulin resistance. Therefore, using a compound that can eliminate or reduce this disorder can play a role in treatment. Antioxidant effects of Propolis and positive effects of chitosan nanoparticle on reproductive system have been suggested in some reports. The aim of current study was to evaluate the effects of chitosan-propolis nanoparticle on Estradiol valerate induced PCOS model of rats.
Materials and Methods: Subcutaneously injection of estradiol valerate (single dose) was used to induce PCOS in rats, followed by oral administration of 500 mg/kg chitosan-propolis nanoparticle for 42 days. Rats were divided into 4 groups; control, PCOS, metformin (PCOS and 150 mg/kg metformin), and chitosan-propolis nanoparticle (PCOS and chitosan-propolis nanoparticle administration, 500 mg/kg) groups. All of the animals were subjected to serum farctors analysis and histopatho-

logical study of ovaries
Results: The body weight and ovarian morphology had been improved and the serum biochemical parameters including esretogen, progestron, vitamin D, calcium, insulin resistance index were reversed after chitosan-propolis nanoparticle intervention.
Conclusion: Data suggested chitosan-propolis nanoparticle could serve as an effective treatment against PCOS-associated insulin resistant. Insulin regulating of propolis extract and increasing bio-distribution properties of chitosan nanoparticle might play a fundamental role in its mechanism of protective effects in the ovarian tissue of PCOS animals.
Keywords: Chitosan Nanoparticle, Estradiol Valerate, Polycystic Ovary Syndrome, Propolis

P-40: Hydroxytyrosol Improves The Parameters of Rooster Sperm After Freezing-Thawing

Kargari M[1*], Sharafi M[1], Karimi Torshizi MA[1], Hezavehei M[2], Zanganeh Z[1]

1. Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: m.sharafi@modares.ac.ir

Background: Cryopreservation of sperm in order to preserve genetic resources is one of the most important reproduction techniques in the livestock and poultry industry. Semen cryopreservation leads to cold stress, reactive oxygen species (ROS) production and increased oxidative stress that causes structural and biochemical damage and imbalance of oxidant and antioxidant in sperm.
Materials and Methods: In this study, we evaluated the effect of the hydroxytyrosol (HT), as an antioxidant, at the levels of 0, 25, 50 and 100 µg on the thawed rooster sperm. Semen samples were collected twice a week from 10 roosters whose age at the beginning of sampling was 29 weeks. After freezing-thawing, sperm parameters including total motility, progressive motility, viability, morphology, membrane integrity and malondialdehyde level of sperm were measured.
Results: The results showed that the 25 and 50 µg of HT had the highest percentage of total motility (51.01 ± 2.83 and 50.15 ± 2.83, respectively) and progressive motility (42.74 ± 1.89 and 40.15 ± 1.89, respectively), as well as membrane integrity (48.00 ± 2.83 and 46.75 ± 2.83, respectively) and sperm viability (53.00 ± 2.80 and 52.50 ± 2.80, respectively) compared to the other groups ($P<0.05$). However, HT could not significantly improve sperm morphology and lipid peroxidation.
Conclusion: Our results showed that HT as an antioxidant in medium doses improved the quantitative and qualitative parameters of rooster sperm after freezing-thawing. These findings suggest that the presence of HT could mitigate ROS concentration, preventing the negative impact of moderately elevated ROS concentrations on the sperm movement. However, the decrease in malondialdehyde levels was not significant compared to the control group.
Keywords: Cryopreservation, Hydroxytyrosol, Rooster, Sperm

P-41: The Protective Effect of Quercetin on The Histopathological and Biochemical Changes of The Male Reproductive System against Exposure to Crude Oil Vapor in Wistar Rats

Khazaeel K[1*], Sadeghi A[1], Tabandeh MR[2], Basir Z[3], Abdul Zahra Daaj S[1]

1. Department of Basic Science, Division of Anatomy and Embryology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
Email: k.khazaeil@scu.ac.ir

Background: Toxic compounds in crude oil vapor (COV), including polycyclic aromatic hydrocarbons (PAHs), are associated with adverse effects on reproduction in living organisms. Quercetin (QT) is the most plentiful flavonoid in vegetables and fruits, with antioxidant activities. This study aimed to evaluate the protective role of QT on testicular toxicity induced by COV.
Materials and Methods: Twenty-four adult male Wistar rats were randomly divided into four groups (n=6) including control, quercetin (QT) (50 mg/kg), crude oil vapors (COV), and COV + QT. The inhalation method was used to expose the rats to crude oil vapors for 5 hours daily, and QT was administered orally. After 30 days, the rats were euthanized, then, the testes were removed for gonadosomatic index (GSI), sperm parameters, H&E staining, the activity of the antioxidant enzymes, and apoptotic gene expression assessments.
Results: The COV statistically significantly ($P<0.05$) reduced GSI, sperm count, motility, viability, and sperm normal morphology, histological indexes, and antioxidant enzyme activities than control. Also, COV statistically significantly ($P<0.05$) increased the expression of caspase-3, p-53, and Bax genes and decreased Bcl-2 gene expression. Co-administration of QT + COV caused a statistically significant ($P<0.05$) decrease in Bax gene expression and increased antioxidant enzyme activities, Bcl-2 gene expression, and reproductive parameters than the COV group.
Conclusion: Based on the results of this study, it appears that crude oil vapor causes side effects on male reproduction. Yet, quercetin has the potential to reduce the side effects of crude oil vapor on the male reproductive system.
Keywords: Crude Oil, Oxidative Stress, Quercetin, Sperm Parameters, Testis

P-42: Protective Effect of Quercetin on Fetal Development and Congenital Skeletal Anomalies against Exposure of Pregnant Wistar Rats to Crude Oil Vapor

Khazaeel K[1*], Ali Hussein H[1], Ranjbar R[1], Tabandeh MR[2], Amir Salman AJ[3]

1. Department of Basic Science, Division of Anatomy and Embryology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Basrah, , Basrah, Iraq
Email: k.khazaeil@scu.ac.ir

Background: Epidemiological evidence indicates a relationship between maternal exposure to crude oil vapors (COV)

during pregnancy and adverse pregnancy outcomes. Quercetin (QUE) supplementation during pregnancy with anti-inflammatory and antioxidant effects can potentially ameliorate the teratogenic effects of environmental pollutants during pregnancy. This study was aimed to investigate the protective role of QUE on fetal development and skeletal abnormalities caused by exposure of pregnant rats to COV.

Materials and Methods: Twenty-four pregnant Wistar rats were randomly categorized into four groups of control, COV, COV + QUE, and QUE (50 mg/kg). From day 0 to day 20 of pregnancy, the inhalation method was used to expose pregnant rats to COV, and QUE was administered orally. Finally, on day 20 of gestation, the animals were anesthetized and a laparotomy was performed, and then the weight and CRL of the fetuses were determined. Skeletal stereomicroscopic evaluations of fetuses were performed using alcian blue/alizarin red staining method, and the expression of osteogenesis-related genes (Runx2 and BMP-4) was evaluated using qPCR.

Results: This study showed that prenatal exposure to COV significantly reduced fetal weight and CRL, and expression of Runx2 and BMP-4 genes. Moreover, COV significantly increased the incidence of fetal skeletal abnormalities such as cleft palate, spina bifida and non-ossification of the fetal bones. However, administration of QUE with exposure to COV improved fetal bone development and reduced fetal skeletal abnormalities.

Conclusion: Quercetin can ameliorate the teratogenic effects of prenatal exposure to COV by increasing the expression of osteogenesis-related genes.

Keywords: Crude Oil, Developmental Toxicity, Congenital Skeletal Anomalies, Quercetin, Fetus

P-43: Enhancing Embryo Development and Redox State Through Supplementation of Embryo Culture Medium with Hydrogen Sulphide Donor

Khodaei Z[1, 2*], Rohollahi Varnoesfandarani SH[2], Hajian Mi[2], Moradi hajidavaloo R[2], Jafarpour F[2], Nasr-esfahani MH[2]

1. Department of Biology, Faculty of Science and Technology, ACECR Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: jafarpour.farnoosh@gmail.com

Background: The recently discovered gaseous molecule, hydrogen sulphide (H2S), has been found to play a role in various biological functions within the male and female reproductive systems. H2S can be generated through two independent pathways: enzymatic and non-enzymatic, in mammals. The p1rimary source of H2S is L-cysteine, which is converted by three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MPST). Several studies have investigated the regulatory effect of H2S on reactive oxygen species (ROS) production in biological systems, suggesting that H2S acts as a chemical reductant by scavenging free radicals.

Materials and Methods: We formulated a hypothesis based on the reducing properties of H2S, suggesting that supplementing the embryo culture medium with GYY4137, a slow-releasing H2S donor, for a duration of 4 days could potentially enhance the developmental competence of cultured embryos and alter the redox state in resulting blastocysts.

Results: Our findings indicate that the addition of 3 μM GYY4137 to the culture medium resulted in an increased blastocyst yield and improved the quality of the resulting blastocysts, as evidenced by a more favorable distribution of blastomeres between the inner cell mass and trophectoderm. Furthermore, our data revealed a decrease in (ROS) levels and an increase in glutathione (GSH) levels within the blastocysts developed in the presence of 3 μM GYY4137 for a duration of 4 days.

Conclusion: These findings suggest that supplementing the embryo culture medium with GYY4137, a slow-releasing H2S donor, has the potential to enhance embryo development and positively influence the redox state in resulting blastocysts, highlighting the importance of H2S in reproductive processes and its potential application in assisted reproductive technologies.

Keywords: Embryo, Hydrogen Sulphide, GYY4137, Mouse, Redox

P-44: The Effect of Fasting on The TLR4/IRF5/Pro-Inflammatory Cytokines Pathway of Testes of High Fat Diet Treated Rats

Kiia M*, Safakhah A, Ebrahimi Meymand A

School of Biology, Damghan University, Damghan, Iran
Email: maedeh.kiia@gmail.com

Background: Previous studies have well shown that a high-fat and high-cholesterol diet reduces the quality of semen by triggering the inflammatory mechanism. It has been found that restricted calorie intake, may reduce the pro-inflammatory cytokines produced from visceral adipose tissue and increase the anti-inflammatory adipokines from that. In this study, our aim is to investigate the effect of one month of fasting on the signaling pathway of TLR4/IRF5/Pro-inflammatory cytokines and also inflammation in the testis of rat treated with high-fat diet(HFD).

Materials and Methods: Eighteen 6-week-old male rats (Sprague-Dawley) with an average weight of 180-200 g were randomly divided into three groups (6 rats in each group): control group, They were fed with a standard diet, for 16 weeks. HFD group, fed with HFD, consisting of 5.3 kcal/g of energy, 20% protein, 36% carbohydrate, 40% fat, 1.25% cholesterol plus 23.1 g/L D-fructose and 18.9 g/L d-glucose for 16 weeks. In the treatment group, mice were fed with a (HFD), for 16 weeks, while from the twelfth week they were subjected to 12 hours of fasting from 9 pm to 9 am for 30 days.

Results: The results showed that fasting can improve testicular morphology, testicular weight, epididymal weight, seminal vesicle weight, prostate weight and sperm duct weight ($P<0.05$). In addition, quantitative and qualitative indicators of sperm, the speed of sperm transfer in the tail of the epididymis, the speed of the transfer of sperm in the body of the epididymis, the number of sperm in the testis, the number of sperm in the tail of the epididymis, the number of sperm in the body of the epididymis, sperm motility, morphology Sperm, hormonal profile, lipid profile, and glucose-related indices were significantly improved in the fasting group compared to the HFD group. Also, our results showed that one month of fasting can reduce the expression of inflammatory signaling pathway genes TLR4, Myd88, Irf5, Mcp-1, TNF-α, IL-1β and IL-6 and the expression of anti-inflammatory cytokine IL-10 increase in testicular tissue ($P<0.05$).

Conclusion: The results of the present study clearly showed that fasting along with food restriction can be used as a thera-

peutic strategy to improve the state of reproductive indicators in obese men.
Keywords: Fasting, Inflammation, Male Infertility, Obesity

P-45: Impact of Methylglyoxal Exposure on Oocyte Development and Redox Balance

Mokhtari Mobarakeh S[1*], Jafarpour F[2], Rahimi M[2], Rouhollahi Varnosfaderani SH[2], Ronasi SH[1], Mahdavi AH[1], Nasr-Esfahani MH[2]

1. Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: jafarpour.farnoosh@gmail.com

Background: Dicarbonyl metabolites are formed through nonenzymatic glycoxidation reactions induced by the nucleophilic addition of free amino groups from proteins, lipids, or nucleic acids to the carbonyl groups of monosaccharides. The most reactive dicarbonyl metabolite in physiological systems is methylglyoxal (MGO). Accumulation of MGO is associated with various pathological conditions such as obesity, polycystic ovarian syndrome (PCOS), diabetes, and aging. It has been demonstrated that MGO disrupts the reduction-oxidation state in biological systems and triggers a series of inflammatory responses. Limited studies have assessed the effect of MGO on the developmental competence of oocytes and growing embryos during preimplantation development.
Materials and Methods: In this study, our aim was to investigate the effect of exposing immature mouse cumulus oocyte complexes (COCs) to MGO during *in vitro* maturation on the developmental competence of challenged oocytes in terms of maturation, pronucleus formation and blastocyst rates. Additionally, we assessed the quality of derived blastocysts in terms of blastomere allocation to the inner cell mass (ICM) and trophectoderm (TE). Furthermore, we evaluated the levels of reactive oxygen species (ROS) and glutathione (GSH) in the resultant blastocysts.
Results: Our findings revealed that exposure of immature COCs during IVM to 75 and 150 μM MGO significantly impaired maturation, pronucleus formation, and blastocyst rates compared to 0, 20, and 40 μM MGO. Furthermore, we observed a significant decrease in the number of TE and ICM cells compared to 0, 20, and 40 μM MGO. Finally, the redox state of blastocysts derived from COCs that exposed to 75 and 150 μM MGO was disrupted, characterized by elevated levels of ROS production and reduced GSH content.
Conclusion: These findings contribute to our understanding of the detrimental effects of MGO on oocyte development and highlight the importance of maintaining proper redox balance during early embryonic stages. Further research in this area is warranted to elucidate the underlying mechanisms and explore potential interventions to mitigate the negative impact of MGO on reproductive outcomes.
Keywords: Methylglyoxal, Mouse, Oocyte, Redox Balance

P-46: Evaluation of Hydroalcoholic Extracts of Rosemary and Black Seed on Oxidative Stress in Cyslophosphamid-Induced Premature Ovarion Failure in NMRI Mice

Mousavizadeh Mobarakeh Z[*], Zeinali H, Azarnia M

Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: za.mousavi93@yahoo.com

Background: Premature ovarian failure (POF) is a disorder of adult women. POF can happen due to chemotherapy, radiation therapy, genetic factors, etc. One of the mechanisms of POF is oxidative stress and as a result follicular atresia, for this reason, we investigated the effect of hydroalcoholic extracts of two plants with antioxidant properties, rosemary (Rosmarinus officinalis L.) and black seed (Nigella sativa), in the POF animal model.
Materials and Methods: POF was induced by 20 mg/kg body weight of cyclophosphamide. Mice were randomly divided: control, model, treatment1: POF + rosemary (40 mg/kg), treatment2: POF + black seed (100 mg/kg), treatment3: POF + rosemary + black seed. 14 days after the treatment, evaluations were done.
Results: The lowest amount of superoxide dismutase and glutathione in the model group and the highest level among the treatment groups was observed in treatment 3. The level of malondialdehyde in the model group increased significantly compared to the control group (310.54 ± 10.06 vs. 150.07 ± 0.04) and decreased in treatment 1, 2 ,3 (203.45 ± 1.44 vs 188.84 ± 4.12 vs 180.96 ± 3.3).
Conclusion: The greatest improvement in oxidative stress was observed in group 3. The observed effects are probably related to the antioxidant and anti-inflammatory properties of the mentioned extracts, and simultaneous treatment with two extracts increased these effects.
Keywords: Black Seed, Cyclophosphamide, NMRI Mice, Premature Ovarian Failure, Rosemary

P-47: Effect of Niacin on *In Vitro* Fertilization of Vitrified Bovine Oocytes

Pakniat Z[*], Azari Mi, Farahmand Dasht Arjaneh F, Eshghi D

Department of Animal Reproduction, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Email: zohre.pakniat72@gmail.com

Background: Today, oocyte freezing has become an essential part of assisted reproductive technologies and has a major impact on the management of reproductive programs. Various protocols such as slow freezing, fast and ultra-fast freezing have been used but so far, no satisfactory results have been obtained. One of the important factors that causes cell damage during the freezing process is free radicals (ROS). Niacin is a broad-spectrum lipid-modifying agent that has potent antioxidant properties and reduces the production of lipid peroxidation. The purpose of the present study was to investigate the effect of addition of Niacin to oocyte maturation media on bovine *in vitro* fertilization after oocyte vitrification.
Materials and Methods: Immature cumulus-oocyte complexes were cultured in tissue culture medium-199 maturation media supplemented with or without 1 mM niacin under a standard *in vitro* culture condition. After 22 hours of culture, matured cumulus -oocyte complexes in both groups were frozen using a standard vitrification procedure. After one week, oocytes were warmed in two steps and evaluated for fertilization rate by aceto-orcein staining. Also mitochondrial distribution were assessed in all groups.
Results: The results indicated that in the control group, the fertilization rate was significantly higher than the cryopreservation

groups (P≤0.05). Between vitrified groups, this difference was statistically close to significant (P=0.08), which could be a sign of the positive effect of niacin added to the culture and freezing medium. Although the uniform distribution of mitochondria in the oocytes of the studied groups was not statistically different, but the peripheral distribution in the CV group was significantly higher than the control group, which could be due to cytoskeletal damage during Freezing and non-displacement of mitochondria to the center.
Conclusion: In conclusion niacin could improve the tolerance of bovine oocytes to vitrification.
Keywords: Bovine, IVF, Niacin, Oocyte, Vitrification

P-48: Effect of Addition of Niacin to Washing and Oocyte Maturation Media on Bovine *In Vitro* Fertilization

Pakniat Z*, Azari Mi, Kafi M, Salehi Kallate Borj A, Eshghi D

Department of Animal Reproduction, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Email: zohre.pakniat72@gmail.com

Background: The high amount of oxygen free radicals in culture medium is a limiting factor for the normal maturation and fertilization of bovine oocytes. Niacin, Nicotinic acid, is a broad-spectrum lipid-modifying agent that has potent antioxidant properties. The purpose of the present study was to investigate the effect of addition of Niacin to washing and oocyte maturation media on bovine *in vitro* fertilization.
Materials and Methods: Good quality immature cumulus-oocyte complexes (COCs) were washed using a Niacin-added washing media and then cultured in TCM-199 media either supplemented with 1 mMol niacin (treated group) or with no addition of niacin (control group) under a standard *in vitro* oocyte maturation system. After 24 hours of culture, the matured Niacin treated COCs were divided into two groups: in one group, matured COCs were fertilized using fertilization medium supplemented with 1 mMol Niacin and the other group of matured COCs were fertilzed using a Niacin free fertilization media. After 18-20 hours, the presumbly zygotes were stained with aceto-orcein and then they were examined for the pronuclei formatiom using an optical microscope. The study was performed in seven independate replicates.
Results: The results of the present study showed that addition of Niacin to the washing/maturation and fertilization media can increase fertilization rates as compared to the control groups (72.7 and 71.3 vs. 61.0%, P<0.05).
Conclusion: The results of the present study showed that addition of Niacin to the washing/maturation and fertilization media can increase fertilization rates.
Keywords: Cows, *In Vitro* Fertilization, Niacin, Oocyte Maturation

P-49: The Effect of The Oral Administration of Nigella Sativa on Quantitative and Qualitative Parameters of Goat Sperm in Liquid Storage

Piri M*, Ghaedi S[1], Hajian M[2], Tanhaee Vash N[2]

1. Department of Animal Sciences, Isfahan University of Technology, Isfahan, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: m.hajian@royan-rc.ac.ir

Background: In recent years, wide utilization of herbal drugs has encouraged scientists to determine their impressive effects on health. On the other hand, the important reason for fertility reduction during sperm storage is the formation of lipid peroxidases in the presence of oxygen radicals. Since Nigella sativa has many uses including infertility in traditional medicine. Therefore, the purpose of this study is to investigate the effect the oral administration of 0.5% Nigella Sativa on quantitative and qualitative parameters of goat sperm in liquid storage.
Materials and Methods: Six adult goats were used in this study, which were divided into two groups: control group (normal feed) and treatment group (normal feed + 0.5% black seed). The goats were fed with these feeds for two months. Semen of each goat was collected by artificial vagina once every three days and after determining the characteristics, they were evaluated. The parameters related to sperm quantity were evaluated after sampling, but the parameters related to sperm quality were evaluated in time intervals of 0, 24, 48, and 72 hours. The volume of the ejaculates was measured in a conical tube, graduated at 0.1 ml intervals, and the ejaculate concentration was measured using a haemocytometer. The percentage of live sperm in the sample was determined utilizing a nigrosin–eosin staining, Sperm motility was by visual assessment, damaged sperm DNA with Acridine orange staining, reactive oxygen species using H2DCFDA staining, Lipid peroxidation by BODIPY and sperm plasma membrane integrity measured by HOST tests.
Results: The results of this study showed that the oral administration of 0.5% Nigella Sativa significantly improved sperm quantitative parameters (semen volume and number of sperm per milliliter) as well as in qualitative parameters compared to the control group (P<0.05).
Conclusion: Based on our results, oral administration of 0.5% Nigella Sativa may increase quantitative and qualitative parameters of goat sperm in liquid storage.
Keywords: Antioxidant, Liquid Storage, Nigella Sativa, Sperm

P-50: Effects of Nano Magnetic Graphene Oxide (MGO) on *In Vivo* Maturation of Oocyte in NMRI Mice

Rahimi Fathkouhi M*, Foroutan T

Department of Animal Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Email: Mitrarahimi34@yahoo.com

Background: Fe_3O_4 super paramagnetic material and graphene oxide (GO) are a good candidates for some applications such as drug delivery. According to the report by the World Health Organization report, 10-15% of couples suffer from infertility, and the use of novel assisted reproductive technologies has attracted increasing interest. In modern *in vitro* fertilization protocols, the use of gonadotropins injection helps to obtain a larger number of oocytes in ovulation. In but in addition to the side effects and the heavy costs, using high doses of hormones may be risky. Therefore, the use of substances that increase the effectiveness of the drug at the appropriate dose can be important. The aim of the present study was present study aimed to investigate the effects of magnetic graphene oxide (MGO) on *in vivo* maturation of mouse oocytes.
Materials and Methods: 30 female NMRI mice (6-8 wk., 25 ± 4gr) as the case group were treated with intraperitoneal (I.P) injection of MGO mixed with Pregnant mare serum gonadotro-

pin (PMSG) and after 12 hours human chorionic gonadotropin (HCG) Hormones was injected. In the other hands, 30 female NMRI mice as the control group were ovarian stimulated without MGO. The number of metaphase II (MII) oocytes obtained from the left fallopian tubes was were counted in each group. Also, immunocytochemical staining of glutathione (GSH) and Reactive oxygen species (ROS) were performed.
Results: The results showed that using MGO in ovarian superovulation increases the number of MII oocytes obtained from the fallopian tube ($p<0.01$). The expression of glutathione GSH of MII oocytes increased in the treated animals ($p<0.001$), and the expression of ROS did not show a significantignificantly increase.
Conclusion: It could be concluded that MGO can increase the efficiency of superovulating hormones due to increased the adsorption of serum proteins and hormones.
Keywords: Fe_3O_4, Graphene Oxide, IVF, Oocyte Maturation

P-51: Field-Assisted Cryopreservation (FAC) of Mouse GV Oocytes Improves *In Vitro* Fertilization Rate and Embryo Development to The Blastocyst Stage

Rahmati Ghezeljeh F[1, 2*], Baniasadi F[1], Rajabi Maham H[2], Fathi R[1]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Medical Ethics and Law, Reproductive Biomedicine ACECR Research Center, Royan Institute for Reproductive Biomedicine, Tehran, Iran
3. Faculty of Science, Biology Department, Shahid Beheshti University, Tehran, Iran
Email: rfathi79@royaninstitute.org

Background: Oocyte freezing is one of the important methods of preserving women's fertility and is a necessity of ART and increases the efficiency of *in vitro* fertilization (IVF) treatment. Since oocyte freezing causes releasing of the cortical granules and hardening of zona pellucida, as a result the rate of and IVF cleavage ability of the produced embryos are decreased. Static magnetic field (SMF) as time-independent field can assist the embryos to rescue from obstacles in the way of blastulation.
Materials and Methods: Immature oocytes were collected from 6-8-week-old NMRI mice and divided into three groups: fresh oocytes, vitrified oocytes, and vitrified oocytes under a magnetic field. In the freezing group with the help of a magnetic field, immature oocytes after washing in equilibrium solution for 5 minutes were placed in a drop of the same medium in the center of mT20 static magnetic field. Next, the oocytes were washed in a vitrification solution for less than 1 minute and transferred on a cryotop, and stored in liquid nitrogen. To warm the oocytes, they were washed in W1 solution for less than 1 minute. In the following, they were washed in W2 and W3 solutions, respectively, and in each stage after washing, they were placed in the center of a 20mT magnetic field for 3 minutes. For *in vitro* maturation, the oocytes were transferred to the IVM environment, and after 13-15 hours, the mature oocytes were transferred to the IVF environment, and the sperms were added to the drops. After 6-8 hours, 2PN embryos were transferred to SAGE medium, and cleavage was followed until blastocyst stage.
Results: The rate of IVF, embryo cleavage, and blastocyst rate decreased in the vitrified group compared to fresh oocytes. In the vitrified group under the magnetic field, the IVF, embryo cleavag and blastocyst rates increased compared to the vitrified group.
Conclusion: The use of this intensity of constant magnetic field to vitrification of immature mouse oocytes by increasing the cell membrane resistance and reducing the cryopreservation damages, oocytes quality was improved and then IVF and embryo cleavage ability to the blastocyst stage were enhanced.
Keywords: Blastocyst, *In Vitro* Fertilization, Mouse Oocyte, Static Magnetic Field, Vitrification

P-52: Differential Effects of Punicic Acid Supplementation on Developmental Competence of Mouse Oocytes: Cumulus Cell Dependency in *In Vitro* Maturation

Ronasi SH[1*], Rouhollahi Varnosfaderani SH[2], Rahimi Andani M[2], Mokhtari S[1], Mahdavi AH[1], Jafarpour F[2], Nasr-Esfahani MH[2]

1. Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: jafarpour.farnoosh@gmail.com

Background: Assisted reproductive techniques (ARTs) are essential approaches for fertility preservation in animal biotechnology as well as in humans. Despite their widespread use worldwide, these techniques can disrupt the microenvironment, potentially compromising the quality of growing embryos during both pre-implantation and post-implantation development. One of the main imbalances in the *in vitro* microenvironment is the redox state, which can lead to oxidative stress and impair the developmental potential of embryos. In light of this, one current approach to address the imbalanced redox state is the supplementation of culture media with antioxidants. A growing body of evidences have shown that polyunsaturated fatty acids (PUFAs) have new insights into health profit. One of the isomers of conjugated linolenic acid (CLnA) – as a subset of PUFAs - is Punicic acid (P.A.), which is mainly found in pomegranate seed oil. CLnAs have been reported to have several beneficial effects including antioxidant and anti-inflammatory effects.
Materials and Methods: In this study we aimed to clarify the effect of treatment of immature mouse oocytes either enclosed with cumulus cells (COCs) or without cumulus cells (Dos) with P.A. during *in vitro* maturation (IVM) on developmental competence including maturation, pronucleus formation and blastocyst rates. Furthermore, the level of ROS production and also of GSH was assessed in treated matured oocytes.
Results: Our findings indicate that the supplementation of IVM medium with different concentrations of P.A., including 0, 0.5, 10, and 100 µM, did not enhance various aspects of the developmental competence of COCs compared to the control group. Furthermore, the levels of ROS and GSH remained unchanged across all P.A. concentrations. Interestingly, we observed that 0.5 µM P.A. significantly improved the maturation, pronucleus formation and blastocyst rates of DOs. In addition, 0.5 µM P.A. decreased the ROS level and increased the GSH level as compared to control group.
Conclusion: These findings suggest that while P.A. supplementation did not improve the developmental competence of COCs, it showed promising effects on DOs. These findings highlight the importance of cumulus cells in mitigating the oxi-

dative stress in oocytes. Further research is needed to explore the potential mechanisms underlying these effects in oocytes during ART procedures.
Keywords: Cumulus Cells, Mouse, Oocyte, Oxidative Stress, Punicic Acid

P-53: Protection of Doxorubicin -Induced Spermatotoxicity by Pentoxifylline in Mice

Shafiei Z*, Mahmoodi M

Department of Biology, Arak University, Arak, Iran
Email: Zshafie373@gmail.com

Background: Doxorubicin (DOX) is a broad-spectrum chemotherapeutic drug widely used in the treatment of a variety of cancers. Although DOX is considered a very efficient chemotherapeutic drug, it also kills healthy cells, especially those under rapid and constant proliferation, such as the male germ cells and lead to infertility. It acts by generating reactive oxygen species in target cells. This study was carried out to investigate the effects of pentoxifylline (PTX), with antioxidant and anti-inflammatory activities, against sperm parameters changes due to DOX treatment in mice.
Materials and Methods: Twenty-four male NMRI (Naval Medical Research Institute) mice were assigned randomly into four groups (n = 6): control, DOX (2mg/kg/weekly), PTX (100mg/kg/daily) and PTX+ DOX. After 35 days intraperitoneal treatment, Sperm samples were collected from cauda epididymidis and used to assess count, motility, viability, morphology, tail length, DNA damage (using acridine orange and aniline blue staining techniques) and daily sperm production. The results were analyzed by one-way ANOVA and Tukey's test.
Results: DOX caused significant decrease in sperm count, motility, viability, morphology, tail length and daily sperm production along with elevated sperm abnormality in comparing to the control group ($P<0.001$). These negative effects were ameliorated following the intervention with PTX. There was no significant difference in sperm DNA damage in DOX treatment groups compared to the control group.
Conclusion: Our results suggest that PTX with its antioxidant properties, can reduce the toxic effects of DOX and improve sperm parameters.
Keywords: Doxorubicin, Mice, Sperm, Pentoxifylline

P-54: The Effect of Estradiol on *In Vitro* Maturation of Immature Oocytes in Premature Ovarian Failure Induced by Cyclophosphamide in NMRI Mice

Sohani Y*, Zeinali H, Azarnia M

Department of Biology, Laboratory of Histology and Embryology, Kharazmi University, Tehran, Iran
Email: yas1375.so@gmail.com

Background: Premature ovarian failure (POF) can occur due to chemotherapy, radiation therapy, genetic factors, etc. In affected people, the follicular function is completely or partially lost. Currently, there is no definitive treatment for this disease, but hormone therapy, egg donation, and cell therapy can be a solution. In this study, the effect of estradiol on *in vitro* maturation (IVM) of mouse oocytes with cyclophosphamide-induced POF was investigated.
Materials and Methods: In order to induce POF model, 8-10-week-old NMRI female mice received cyclophosphamide 20 milligram/kilogram (mg/kg) body weight intraperitoneally for 21 days. Immature oocytes were extracted and randomly divided into the following groups: sham (no estradiol) and treatment (maturation medium containing 1.5 micrograms/milliliter (µg/ml) estradiol). The percentage of oocytes in different maturation stages was analyzed by SPSS software and $P<0.05$ was considered as a significant level.
Results: The percentage of mature oocytes in the maturation medium containing 1.5 µg/ml of estradiol was higher compared to the sham group. The lowest percentage of germinal vesicle breakdown (GVBD) and mature oocytes, and the highest percentage of immature and degenerated oocytes were observed in the sham group.
Conclusion: Probably, estradiol can be an effective factor on *in vitro* maturation of mouse oocytes with POF and increase their maturation percentage.
Keywords: Cyclophosphamide, Estradiol, *In Vitro* Maturation, Oocyte, Premature Ovarian Failure

P-55: The Effects of Different Levels of N-Acetyl Cysteine in Two Equilibration Periods on The Rooster Sperm Parameters after Freezing-Thawing

Zanganeh Z[1*], Sharafi M[1], Halvaei I[2], Hezavehei M[3], Rahimi SH[1]

1. Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Anatomical Sciences, 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: m.sharafi@modares.ac.ir

Background: Oxidative stress and reactive oxygen species (ROS) production are the important reasons for decreased sperm function during the cryopreservation process. Using of antioxidants can improve the cryo-survival of sperm act as a protection approach. Moreover, equilibration time is an important factor that could affect on the quality of thawed sperm. The aim of this study was to investigate the effects of N-acetyl cysteine (NAC) as an antioxidant and two equilibration times on the quantitative parameters of rooster sperm after freeze-thaw.
Materials and Methods: Semen samples were collected from 10 roosters. Then samples were pooled and divided into four equal parts to be diluted with Beltsville extender containing different concentrations of NAC as following groups: 0, 0.1, 1 and 10 mM/ml. Each of these groups was divided into two parts to undergo 2- and 4- hour cooling equilibration periods. In the first phase, motility parameters, plasma membrane integrity and sperm viability were evaluated. According to the results, 3 selected groups NAC-0.1-2 hours, NAC-1-2 hours, NAC-1-4 hours were evaluated for sperm functional parameters such as lipid peroxidation, mitochondrial membrane potential, apoptosis and intracellular ROS concentration.
Results: A higher percentage of motility parameters, plasma membrane integrity and sperm viability were observed in the NAC-1-2 hours and NAC-1-4 hours groups. Moreover, these groups significantly reduced the amount of apoptosis and the level of intracellular ROS.
Conclusion: NAC-0.1 and NAC-1 in the 2-hour equilibration

period caused a significant improvement in the quantitative and qualitative parameters of rooster sperm, which could be due to the success of this antioxidant in reducing intracellular ROS and oxidative stress.
Keywords: Equilibration Time, N-acetyl Cysteine, Rooster, Semen

P-56: Protective Effects of Resveratrol on The Thawad Rooster Semen During Different Equilibration Times

Zanganeh Z[1*], Sharafi M[1], Halvaei I[2], Hezavehei M[3], Rahimi SH[1]

1. Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Anatomical Sciences, 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: m.sharafi@modares.ac.ir

Background: Forasmuch as the rooster sperm has low level of cytoplasmic antioxidants, are very susceptible to increased oxidative stress during cryopreservation. Also equilibration time during cryopreservation can effect on the post-thaw sperm survival. Therefore, we evaluated the effect of resveratrol (RSV) as an antioxidant and two equilibration times on the parameters of thawed rooster sperm.
Materials and Methods: Twice a week, semen samples collected from 10 roosters and diluted in a Beltsville extender, then supplemented with 4 levels of RSV (0, 0.1, 1, and 10 µm/ml), and each level was divided into two parts to undergo equilibration periods (2- and 4-hour). In the first phase, motility parameters, sperm viability and plasma membrane integrity were evaluated. According to the results of the first phase, RSV-1-2hr, RSV1-4hr and RSV10-4hr groups were selected to evaluate lipid peroxidation, apoptosis rate, mitochondrial membrane potential (MMP) and intracellular reactive oxygen species (ROS) concentration.
Results: RSV-1-2 hour and RSV1-4hr groups significantly improved the evaluated parameters in the first phase. RSV-1-2 hour group significantly reduced the level of malondialdehyde compared to other groups. RSV-1-2 hour and RSV-1-4 hour groups significantly reduced the apoptosis and intracellular ROS.
Conclusion: RSV-1-2hr and RSV1-4hr improved the efficiency of the thawed rooster sperm, which indicates the dose-dependent effect of RSV on the values of sperm kinetic variables. Because RSV can reduce lipid peroxidation in cells, it is presumed that the positive effect of RSV on quantitative and qualitative parameters of rooster sperm subsequent to thawing was due to its protective functions when there is greater than optimal ROS generation and lipid peroxidation.
Keywords: Resveratrol, Rooster Sperm, Cryopreservation, Equilibration Time

Embryology

P-57: The Effect of Carob Pod Extract on Ram Sperm Motility after Freezing

Asgari M[*], Khodaie-Motlagh M, Kazemi-Bonchenari M

Department of Animal Sciences, Faculty of Agriculture and Natural Resource, Arak University, Arak, Iran
Email: Mohsen.asgari20.ma@gmail.com

Background: Sperm cells have a mechanism of enzymatic and non-enzymatic antioxidant is but in the process, the Internal dilution ratio dropped antioxidants and for the treatment of sperm need to add antioxidants of foreign origin. Antioxidants are compounds the synthesize of free radicals, especially reactive oxygen species 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. The purpose of this study determine the antioxidant effect the carob pod extract on sperm motility rams Farahani 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 contains 12 to 16 hard seeds. Carob is native to mediterranean regions and is found in the south of syria, India and most of the mediterranean areas as well as in california. It grows wildly in Shapoor, Fars, Iran. Carob pods have been used in many countries as an antioxidant in different foods, as thickener, stabilizer or flavourant in food applications, in ethanol production,in the production of cosmetics, in animal nutrition, in lactic acid production and in medical applications etc. The carob pod as a potential source of natural antioxidants to be considered. Aantioxidant activity carob regarding 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, and penicillin (100,000). After dilution of sperm zero concentration as a witness and treatments of 0.05 ml and 0.1 ml of carob pod extract the diluent containing the sperm was added.
Results: The achieved results showed that extract carob pods of 0.05 and 0.1 had a significant effect on sperm protection ability and motility.
Conclusion: Results indicated that adding 0.05 and 0.1 mL of carob pod extract peel to Tris-based extender was beneficial in storage in sperm Farahani ram breed after freeze-thawing.
Keywords: Antioxidant, Carob Pod Extract, Farahani Ram, Sperm Motility

P-58: Effect of Fenoprofen on Serum Levels of Nitric Oxide (NO) in Female Rats With PCOS

Amin F *, Rajabi-Maham H , Azizi V, Hosseini A

Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
Email: cellularreproductive@gmail.com

Background: Polycystic ovary syndrome (PCOS) is a common worldwide syndrome among women of reproductive age, increasing their infertility risk. Evidence shows that PCOS is a chronic inflammatory disease. This experiment aims to evalu-

ate the effect of the anti-inflammatory drug fenoprofen on the serum level of nitric oxide NO.
Materials and Methods: To do this work, we categorized 25 Wistar rats into five experimental groups, including the control, PCOS, and treatment group. We induced PCOS model by intramuscular injection of estradiol valerate in the estrus cycle to the experimental groups. After one month, we treated them with intraperitoneal(ip) injections in 5, 10, and 20 doses of fenoprofen for one week. After the treatment, we anesthetized rats by ip injection of ketamine and xylazine, and blood was collected from their hearts, centrifuged, and the serum was separated. We checked the serum sample's NO concentration using the NO conventional kit.
Results: The serum level of NO in the PCOS group is higher than in comparison with the control group ($P<0.05$). Also, treatment groups comparison with the PCOS group ($P<0.05$)
Conclusion: In conclusion, in this experimental study treatment with fenoprofen showed a significant effect in rats with PCOS.
Keywords: Fenoprofen, Nitric Oxide, PCOS, Rat

P-59: The Effect of Green and Chemically Synthesized Copper Nanoparticles on the Oocyte Maturation and Viability

Bagheshzadeh P[1, 2*], Amini E[1], Baniasadi F[2], Tavana S[2], Mohammadikish M[3]

1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Inorganic Chemistry, Faculty of Chemistry, Kharazmi University, Tehran, Iran
Emails: elaheh.amini@khu.ac.ir; s.tavana@royan-rc.ac.ir

Background: *In vitro* maturation (IVM) of oocyte is a method of infertility treatment. Oxidative stress resulting from the imbalance between reactive oxygen species (ROS) and antioxidants is the main challenge in this technique, which reduces oocyte viability and maturation. Therefore, this problem can be moderated by using exogenous antioxidants, including green copper nanoparticles (Cu NPs) that have antioxidant properties.
Materials and Methods: Immature oocytes (GV oocytes) from 6-8 weeks NMRI mice were collected and divided into 4 groups labeled as control, Cu NPs synthesized by the green method through rosemary aqueous extract (G-A-NPs) or using rosemary hydro-alcoholic extract (G-H-NPs) and Cu NPs synthesized by chemical method (Ch-NPs). After 18 hours of cultivation, the rate of matured oocytes (MII oocytes), nuclear maturation of MII oocyte (using Hoechst staining), apoptosis, necrosis and survival of MII oocytes (using Anex-PI staining) were evaluated.
Results: The maturation rate of the oocytes of the control group, G-H-NPs and G-A-NPs showed a significant increase compared to the Ch-NPs group, but the maturation rate of the oocytes of the G-H-NPs and G-A-NPs group did not show a significant difference compared to the control group. By binding the Hoechst dye to the DNA of the nucleus of the polar body of the oocytes, the real maturation of the oocyte was confirmed. The survival rate of the Ch-NPs group showed a significant decrease compared to the control group, but the G-H-NPs and G-A-NPs groups did not differ significantly from the control group. A non-significant difference was observed between the 4 groups in terms of primary apoptosis, secondary apoptosis and necrosis.
Conclusion: Copper nanoparticles synthesized by the green method showed better performance in oocyte maturation and survival due to their antioxidant properties and greater safety in synthesis compared to chemical copper nanoparticles.
Keywords: Antioxidant, Copper Nanoparticles, Green Synthesis, *In Vitro* Maturation

P-60: Evaluation of The Effects of Hydroxytyrosol on Human Sperm Parameters during Cryopreservation

Baharsaadi M*, Hezavehei M , Shahverdi AH, Halvaei I

Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, Tehran, Iran
Email: m.bahar69m@gmail.com

Background: Human sperm cryopreservation is a routine procedure in assisted reproductive technology that has detrimental effects on different sperm parameters. It is worthwhile to introduce a suitable antioxidant to reduce reactive oxygen species (ROS) and maintain sperm viability under freezing-thawing stress. Our aim was to evaluate the effects of hydroxytyrosol (HT) as an antioxidant, on human sperm parameters after cryopreservation.
Materials and Methods: 20 normal semen samples were cryopreserved by rapid freezing method with different doses of HT including 0, 50, 100, 150, and 200 µg/mL. The optimum concentration of HT was determined by evaluation of motility parameters (computer-assisted semen; CASA), viability (Eosin-nigrosine stain), DNA integrity (sperm chromatic dispersion test: SCD), reactive oxygen species (DCF and DHE staining, flowcytometry) lipid peroxidation (malondialdehyde, MDA test) and mitochondrial membrane potential (JC1 staining: flowcytometry) of sperm in between different groups.
Results: Sperm motility had an increasing trend in 50 and 100 µg/mL HT in comparison with other groups but the difference was not significant. The highest significant percentage of viability was obtained in 50 and 100 µg/mL HT and also, the lowest significant percentage of DNA fragmentation was in samples treated with 100 1µg/mL HT compared to the other groups ($P<0.05$). However, the level of intracellular reactive oxygen species, lipid peroxidation and mitochondrial membrane potential were not significantly between groups.
Conclusion: Our results showed that HT may have protective effects on viability and DNA integrity of thawed human sperm.
Keywords: Antioxidant, Cryopreservation, Human Sperm, Hydroxytyrosol, DNA integrity

P-61: The Urtica Dioica Improves Structural Disorders in The Testis Of Streptozotocin-Induced Diabetic Male Rats

Bahrami Tapehbour M[1*], Fathi A[2], Iranshahi R[2]

1. Department of Anatomy & Embryology, Faculty of Veterinary Medicine at Lorestan University, Khorramabad, Iran
2. Department of Histology, Faculty of Veterinary Medicine at the Shahid Chamran University of Ahvaz, Ahvaz, Iran
Email: bahrami.m@hotmail.com

Background: Diabetes mellitus can lead to structural disorders in the testis. Urtica dioica has antidiabetic, antioxidant, and

hypoglycemic effects. This study was designed to evaluate the effects of hydro-alcoholic extraction of nettle plant (Urtica dioica) on the morphometric changes in the testis of streptozotocin-induced diabetic male rats.
Materials and Methods: Twenty five male Wistar rats were randomly divided into 5 groups including: control (normal saline), diabetic (normal saline), Urtica dioica (200 mg/kg/day; gavage), diabetic + Urtica dioica (200 mg/kg/day; gavage) and diabetic + Neutral Protamine Hagedorn (NPH) insulin (10 IU/kg/day; subcutaneous). Experimental diabetes was induced by streptozotocin injection (60 mg/kg; intraperitoneal). All groups were treated for 8 weeks. At the end of treatment course, the rats' testis were removed intact immediately after sacrifice for morphometric measuring by caliper. Histological testis sections were also prepared to assess the morphometry of seminiferous tubules and spermatogenic cells via hematoxylin and eosin staining.
Results: Diabetes induction reduced weight and dimensions of the testis, diameters of the epithelium of the seminiferous tubules, and spermatogenesis cell number compared to the control group after 8 weeks ($P<0.05$); while treatment of diabetic rats with Urtica dioica or insulin significantly decreased the structural alterations of the testis and improved the blood glucose level of diabetic rats ($P<0.05$).
Conclusion: The results showed that oral administration of Urtica dioica in diabetic rats improves structural disorders of testis and ameliorates diabetes-induced hyperglycemia.
Keywords: Diabetes Mellitus,Urtica Dioica, Rat,

P-62: *In vitro* Development of Mouse Preantral Follicles in Conventional and Suspension Culture Conditions

Bazgir A[1*], Farokhi F[1], Dalman A[2], Hassani F[2]

1. Departmen of Biology, Faculty of Science, Urmia University, Urmia, Iran
2. Departmen of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: a.dalman@royan-rc.ac.ir

Background: Three-dimensional follicle culture systems have used different strategies to prevent spherical follicles from adhering to the culture wells to provide a more natural environment for oocyte development. The approach used in this research was the droplet method using a petri dish suspension, which avoids the need to transfer follicles daily to prevent them from adhering.
Materials and Methods: Preantral follicles with an average size of 130µm were isolated from the ovaries of two-week-old NMRI mice and cultured for 13 days in a SAGE culture medium with 100mIU FSH along with ITS and FBS in two-dimensional and suspension culture systems. On the 13th day of culture, hCG was added to the culture medium to resume meiosis and maturation of oocytes. Then, the rate of antrum cavity formation and oocyte maturation was evaluated. Immunocytochemical staining for alpha-tubulin was performed to measure the quality of matured oocytes.
Results: There was no difference between the two experimental groups regarding the follicle development and oocyte maturation rate. Still, the morphological evaluation of mature oocytes obtained from culture showed that the oocytes grown in suspension conditions were of higher quality. This higher quality was confirmed by immunocytochemical staining and evaluation of the structure and orientation of the meiosis spindle and metaphase plate chromosomes.
Conclusion: This study showed that the suspension culture system and the SAGE culture medium containing 100 mIU/mL FSH, while maintaining the integrity of the follicle structure, can produce high-quality oocytes regarding nuclear maturation, and cytoplasmic maturation.
Keywords: *In Vitro* Follicle Culture, Suspension Culture, Oocyte Developmental Competence

P-63: The Effect of L-Carnitine on Oocyte Mitochondrial Health And Biomarkers on Cyclophosphamide Induced in Mice

Behi shahreza P[1*], Ahmadpour S[2], Almasi M[1], Seyyed Hosseini E[1], Akhavan Taheri M[1], Moshkdanian G[1]

1. Gametogenesis Research Center, Institute for Basic Sciences, Kashan, Iran
2. Department of Biotechnology, Faculty of Chemistry, Kashan, Iran
Email: gmoshkdanian@gmail.com

Background: Improving oocyte competence during chemotherapy is a contributing factor, which can increase the probability of fertility. Also, the role of cumulus cells in oocyte quality is crucial. Due to the wide use of cyclophosphamide chemotherapy and the adverse effects of this drug on the female reproductive system and fertility rate, this study was designed to evaluate the effect of L-carnitine on oocyte biomarkers genes (Gdf9, Has2, Cx43 and, Cx37) also Sirt3 gene as a factor for mitochondrial health against cyclophosphamide exposure.
Materials and Methods: A total of 60 adult NMRI mice were divided into 4 groups including Control, L-carnitine (LC), Cyclophosphamide (CP), and Cyclophosphamide +L-carnitine (CP+LC). The relative mRNA expression levels of oocyte quality genes including Growth differentiation factor 9 (Gdf9), Hyaluronan synthase 2 (Has2), and mitochondrial Sirtuin 3 (Sirt3) in oocytes, and genes involved in bilateral communication between cumulus cells and between the oocyte and its neighboring cumulus cells including Connexin 37 (Cx37) and Connexin43 (Cx43) were detected by real-time polymerase chain reaction. The 2,7-dichlorofluorescein diacetate staining analyzed the level of intracellular reactive oxygen species (ROS) in oocytes.
Results: Under the influence of L-carnitine Gdf9, Has2, Cx43, and Cx37 were significantly up-regulated ($P\leq0.01$). However, cyclophosphamide significantly reduced the expression of all these genes ($P\leq0.05$). The expression of the Sirt3 gene in the CP group increased significantly compared to other groups ($P\leq0.01$). Analysis of fluorescent images showed that the level of intracellular ROS in the cyclophosphamide group was significantly increased compared to other groups ($P\leq0.01$) while in the L-carnitine group, the level of it decreased significantly ($P\leq0.01$).
Conclusion: L-carnitine as an antioxidant can reduce the destructive effects of cyclophosphamide and enhance the bilateral communications between oocytes and cumulus cells, and finally may lead to an increase in the fertility rate.
Keywords: Cyclophosphamide, Female Infertility, L-carnitine, Gap Junction, ROS

P-64: Assessing The Impact of Advanced Glycation End Products Diet on Sperm Quality in C57BL Mice: A Comparative Study of 5 and 13 Weeks

Darmishonnejad Z[1, 2*], Hassanzadeh V[1], Tavalaee M[2], Kobarfard F[3], Nasr-Esfahani MH[2]

1. Department of Cell and Molecular Biology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Medical Chemistry, Shahid Beheshti School of Pharmacy, Tehran, Iran
Email: z.darmishoun@gmail.com

Background: Accumulation of advanced glycation end products (AGEs) can cause oxidative stress, inflammation, and cell damage, including damage to cell membranes and DNA. To better understand the effects of an AGE diet on sperm quality, we conducted a study comparing the sperm of C57BL mice after being fed an AGE diet for 5 and 13 weeks. Our aim was to establish a model for AGE accumulation in mice and assess the potential impact on sperm quality.
Materials and Methods: This research study involved twenty C57BL mice that were randomly divided into two control groups and two groups fed an AGE diet for 5 and 13 weeks, respectively. Fasting blood sugar (FBS), sperm parameters, and sperm functional tests were compared among the groups. sperm morphology, histone residual, protamine deficiency, and DNA damage. Statistical analysis was conducted using one-way analysis of variance (ANOVA) to compare the study parameters among the four groups. A p-value less than 0.05 was considered statistically significant.
Results: The FBS levels in both the 5-week and 13-week AGE groups were significantly higher than in the two control groups. Among the sperm parameters evaluated, both total motility and progressive motility were significantly lower in the AGE groups for both 5 and 13 weeks compared to the control groups. Only sperm concentration in the 13-week AGE group was significantly lower than its self-control. The mean quality of the sperm function showed a significant reduction in both AGE groups compared to the control groups.
Conclusion: Both the 5-week and 13-week AGE diet groups had a negative impact on sperm parameters and function. However, the 13-week AGE diet group showed a more negative effect on sperm concentration in addition to all the other parameters.
Keywords: Advanced Glycation End Products, Sperm Chromatin, Sperm DNA Damage, Sperm Parameters

P-65: Differential Follicle Count for Investigating The Ovarian Toxicity of Cyclophosphamide in The Rat

Elahi N[1, 2*], Ai J[3], Astaneh ME[2, 4], Makoolati Z[4]

1. Students Research Committee, Fasa University of Medical Sciences, Fasa, Iran
2. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Fasa University of Medical Sciences, Fasa, Iran
3. Department of Tissue Engineering, Tehran, Iran, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Anatomical Sciences, Faculty of Medicine, Fasa University of Medical Sciences, Fasa, Iran
Email: zohreh1438@yahoo.com

Background: Impaired ovarian function following chemotherapy occurs with variable degrees in cancerous women. Cyclophosphamide (CPA) has been widely administered in different concentrations for induction of the ovarian toxicity and for designing premature ovarian failure (POF) in animal models. It has serious side effects on abdominal organs and bone marrow. In this regard, it is essential to find the minimum applicable dose of CPA for ovarian toxicity induction.
Materials and Methods: Ten adult female Sprague Dawley rats (250-300 g, 10–12 weeks old) were divided randomly into two groups. The treatment group was intraperitoneally injected with 50 mg/kg of CPA on the first day and then 8 mg/kg for 14 consecutive days. The control group received no injection. At the end of the induction period, rats were euthanized and ovaries were removed based on the analysis protocol and stereological assessment. Differential follicle categorizations were made exploiting the standard definition of follicle classifications. Image J software was applied to evaluate total follicle numbers.
Results: A significant decrease was observed in primordial, primary, preantral and graaf follicles of administrated rats relative to the control group. Results of differential follicle count in histological assay also showed that graaf and preantral follicles were the most sensitive groups to CPA, followed by primary and primordial follicles (41.36, 68.76, 79.55, and 84.88 % of control, respectively).
Conclusion: Various studies applied variable doses of CPA for ovarian toxicity induction. High doses of CPA have serious side effects and also increase the rate of mortality. Therefore, it is important to select the optimal dose to reduce mortality and induce the toxicity of the ovary at the same time in animal studies. Our findings displayed a remarkable reduction in all types of studied follicles by a CPA dose of 50 mg/kg which can be suggested as an applicable dose for CPA-induced ovotoxicity in research.
Keywords: Cyclophosphamide, Follicles, Ovarian Toxicity, Histology

P-66: Diabetes Mellitus Decreases E-Cadherin in Rat Endometrium at The Time of Embryo Implantation

Haghi M*, Foroutan T

Department of Animal Sciences, Faculty of Biology Science, Kharazmi University, Tehran, Iran
Email: maryamhahi1997@gmail.com

Background: Diabetes mellitus deeply changes some adhesion molecules genes expression such as integrin in several cells. Adhesion molecules play an important role in embryo implantation. Since the rate of pregnancy is reduced in diabetic patients, the aim of the current study was to investigate the endometrial gene expression of E-cadherin as an adhesion molecule in diabetic rat models at the time of embryo implantation.
Materials and Methods: Sixteen rats were randomly divided into 2 groups; control and diabetic group. Immunohistochemistry staining was performed to determine changes in the expression of E- cadherin protein in rat's endometrium. Glucose and insulin hormones of serum in the control and experimental groups were assessed at different times.
Results: The expression of E- cadherin reduced significantly in diabetic rats' endometrium compared with the control group. Also, FSH and progesterone hormone secretion reduced significantly in diabetic rats compared with the control group.
Conclusion: Diabetes mellitus significantly reduced the expression of E- cadherin adhesion molecule, therefore untreated

diabetes could be potentially assumed as one of the preliminary elements in embryo implantation failure.
Keywords: Diabetes Mellitus, Embryo Implantation, E- Cadherin, Endometrium

P-67: Identification Protein-Protein Interaction And Lncrnas Network of PPARs Expression Related Genes With Rat Offspring's Semen Quality Under Dietary Vitamin E Supplementation And Trans-Fatty Acids

Hashemi Karoii D[1*], Shahhoseini M[1, 2]

1. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: d.hashemi.karoii@ut.ac.ir

Background: The ligand-activated nuclear receptor superfamily includes transcriptional factors known as peroxisome proliferator activated receptors (PPARs). They are widely expressed all across the body. There are three main kinds known: PPARa, PPARb, and PPARg. For the metabolism of lipids and glucose, respectively, PPARb and PPARg are essential. The modulation of many forms of inflammation and infertility is another function of PPARs. establishing a connection between the recommended status of vitamin E as a primary therapy option in offspring's semen quality and the PPAR regulation by oxidized vitamin E metabolites. Our recent study demonstrates that vitamin E supplementation can enhance these metrics. While diets high in vitamin E increased the transcription of PPAR genes, diets high in trans-fatty acids decreased the expression of PPAR and PPAR genes.
Materials and Methods: To predict and identify the hub imprinting gene target, we use the lncRNA and protein-protein interaction (PPI) database. With the use of the LncPath and lncGSEA programs, the differential expression of miRNAs and lncRNAs was discovered. PPI and functional enrichment analysis were carried out. After filtering out the hub genes, a molecular docking study was carried out.
Results: We discovered that the four datasets shared 152 PPI and 136 intersected differentially expressed lncRNA. We identified the top 5 hub lncRNAs that strongly influenced these target genes, including LINC02177, LINC01819, LINC00525, and RHOXF1-AS1. Molecular docking specifically anticipated that lncRNA-PPI would be a suitable medication. We identified the top 7 hub PPIs that significantly impacted the target genes TMEM159, NRIP1, FAM120B, NCOA1, NCOA2, FABP5, and ANGPTL4.
Conclusion: The activity of the PPARs protein network in response to dietary vitamin E supplementation and trans fatty acids may also be determined by determining the protein partners of the PPARs.
Keywords: PPAR, Lncrna, Vitamin E, Semen

P-68: Watercress Seed Extract And Its Effect on Fertility in Diabetic Male Rats

Honarmandnia S[1*], Seyed Hosseini E[2], Haddad Kashani H[3]

1. Molecular diagnosis lab , Valfajr Health Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Gametogenesis Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran
3. Anatomical Sciences Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran
Email: s.honarmand83@gmail.com

Background: Diabetes has a major impact on the male reproductive system and can lead to male infertility. Plants have recently emerged as a lucrative source of medicine This study evaluated the potential protective effect of watercress (Lepidium sativum) seed extract on fasting blood glucose (FBS) and then accurately measured epididymal histopathological changes in streptozotocin (STZ)-induced diabetic rats.
Materials and Methods: Fifty male adult Wistar rats were randomly divided into five groups (10 rats in each group). The first group is the placebo control group, which is fed only 0.1 ml of normal saline, and the second group is the diabetic control rats, which are injected intraperitoneally with 60 mg STZ/kg body weight. FBS > 250 mg/dl was judged as diabetes. Group 3 were diabetic rats administered insulin at a dose of 3 U/100 g body weight, and groups 4 and 5 were diabetic rats administered 0.1^{cc} of 200 and 400 mg/kg ethanol extract from lindenia seeds per day received tube feeding. One day after the last gavage, the rats were anesthetized with chloroform.
Results: Adjusted doses of 200 and 400 mg/mL cress seed extract significantly increased epithelial cell height and significantly decreased replacement volume mass and fibromuscular thickness. In addition, the volumetric mass of epithelial cells, fibromuscular cells, lumen, and stroma was significantly reduced. Tubular and luminal diameters remained largely unchanged across groups.
Conclusion: There is no doubt that cress seed extract can be used as a secondary protectant to alleviate the adverse effects of diabetes on the reproductive system in diabetic males.
Keywords: Cress, Diabetes, Streptozotocin, Insulin, Seed Extract

P-69: Investigating The Effect Of Empagliflozin on The Number Of The Spermatogenic, Sertoli And Leydig Cells in The Diabetic Rats

Kiani M[*], Soleimani Mehranjani M, Shariatzadeh Seyed MA

Department of Biology,Faculty of Sciences ,Arak University, Arak, Iran
Email: minakiani335@gmail.com

Background: Empagliflozin has antioxidant properties. An increase in blood sugar causes increase in the level of oxidative stress and a disturbance in the glucose metabolism of the testis. We aimed to investigate the effect of empagliflozin on the number of spermatogenic, Sertoli and Leydig cells in diabetic rats.
Materials and Methods: 18 adult male rats were divided into control, diabetic (streptozotocin (65 mg/kg b.w) and nicotinamide (110mg/kg b.w), intraperitoneal injection) and diabetic+Empagliflozin group (10 mg/kg b.w/day, gavage, 8 weeks). After 8 weeks, IUR sections were prepared from the right testis and after tissue passage, 20 μm thick sections were prepared. spermatogenic, Sertoli and Leydig cells were counted. The data were analyzed using ONE-WAY ANOVA and Tukey's test, and the significance level was $P<0.05$.
Results: A significant decrease in the number of spermatogonia cells, primary spermatocytes, round spermatids, elongated spermatids, Sertoli, and Leydig cells were observed in the diabetic group compared to the control ($P<0.001$). In the diabetic+ Empagliflozin group the average number of spermatogonia

cells and primary spermatocytes (P<0.01), round spermatids (P<0.001), elongated spermatids (P<0.001), Sertoli (P<0.01) increased significantly compared to the diabetic group(P>0.05).
Conclusion: Empagliflozin improves the effects of type 2 diabetes on the number of spermatogenic and Sertoli cells but does not affect the number of Leydig cells.
Keywords: Empagliflozin, Leydig Cells, Spermatogenic Cells, Sertoli Cells, Type 2 Diabetes,

P-70: Investigating The Effect of Myoinositol on Sperm Parameters in Rats With Type 2 Diabetes

Kiani M*, Soleimani Mehranjani M, Shariatzadeh Seyed MA

Department of Biology,Faculty of Sciences ,Arak University, Arak, Iran
Email: minakiani335@gmail.com

Background: The increase in the level of oxidative stress and the decrease in the level of Myoinositol in type 2 diabetes, affect sperm parameters. We investigated the effect of the Myoinositol, as an antioxidant, on sperm parameters in diabetic rats.
Materials and Methods: 18 adult male rats were divided in three groups: control, diabetic (streptozotocin (65 mg/kg b.w) and nicotinamide (110 mg/kg.b.w), intraperitoneal injection) and diabetic + Myoinositol group (300 mg/kg.b.w/day, gavage, 8 weeks). After 8 weeks, semen samples were collected from the epididymis, and after preparing the smear, Eosin-Nigrosin, Diff quick staining was used for the sperm viability and morphology, respectively. Data were analyzed using ONE WAY ANOVA and Tuky's test, and the significance level was P<0.05.
Results: A significant decrease in sperm count, viability, progressive motility, total motility, normal sperm morphology (P<0.001), as well as a significant increase in stationary motility, number of headless sperms and tailless sperms was observed in the diabetic group compared to the control (P<0.001). In The diabetic + Myoinositol group, a significant increase in sperm count (P<0.001), viability and progressive motility (P<0.05), total motility (P<0.01), normal sperm morphology (P<0.001) and also a significant decrease in the stationary motility (P<0.01), number of headless sperms (P<0.05), and tailless sperms (P<0.001) were observed.
Conclusion: Myoinositol protects the sperm against type 2 diabetes by improving sperm count, viability, motility, and morphology.
Keywords: Myoinositol, Sperm, Type 2 Diabetes

P-71: The Effect of Alpha Lipoic Acid on Sperm Parameters During Cryopreservation in The Asthenozoospermic Men

Kohzadi R[1*], Soleimani Mehranjani M[1], Cheraghi E[2], Shariatzadeh MA[1]

1. Department of Biology, Faculty of Science, Arak University, Arak, Iran
2. Department of Biology, Faculty of Science, University of Qom, Qom, Iran
Email: ronak.kohzadi@gmail.com

Background: Asthenozoospermia is one of the most prevalent causes of male infertility. Although cryopreservation is an indispensable part of assisted reproductive centers, it decreases sperm quality. Adding an antioxidant to the cryopreservation medium could be an effective strategy to decrease the adverse effects of cryopreservation. Alpha lipoic acid (ALA) has been described as a potent biological antioxidant. In this study, the effect of ALA supplementation on sperm parameters during cryopreservation of semen samples of asthenozoospermic men was assessed.
Materials and Methods: Thirty semen samples were collected from asthenozoospermic patients who had been referred to the infertility treatment center of Qom University Jihad in 2021. Each sample was divided into 3 groups: Control (fresh), Freeze (treated with cryo-protectant alone), and Freeze+ ALA (treated with cryo-protectant+ 0.5mM ALA solution [8-10]). In the freezing groups, samples were cryopreserved with human sperm freezing medium and rapid freezing method. In each sample, sperm mobility according to WHO criteria, sperm viability using eosin-nigrosin staining and sperm morphology using the Diff Quick kit were assessed. Data were analyzed statistically using the Repeated Measure Analysis method and Bonferroni post-hoc test.
Results: Sperm motility, viability and normal morphology significantly decreased in the Freeze group compared to the Control group (P=0.000). Whereas, in the Freeze+ ALA group a significant increase was observed in these parameters compared to the Freeze group (P=0.000).
Conclusion: Our results showed that ALA ameliorates the adverse effects of cryopreservation on sperm quality in asthenozoospermic men.
Keywords: Asthenozoospermia, Cryopreservation, Alpha Lipoic Acid, Sperm Parameters,

P-72: Fabrication of A Testicular ECM/Gelatin Porous Three-Dimensional Scaffold By Gas-Foaming Technology

Momeni M[1*], Kazemi Ashtiani M[2], Bashiri Z[1], Bagher Z[3, 4], Asgari HR[1], Koruji M[1, 5]

1. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. ENT and Head and Neck Research Center and Department, The Five Senses Health Institute, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
5. Stem Cell and Regenerative Medicine Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: koruji.m@iums.ac.ir

Background: Successful *in vitro* spermatogenesis is crucial to help prepubertal boys and adult male cancer patients. For its study, the use of decellularized testicular ECM to fabricate an optimal scaffold is a well-known tool. Moreover, ECM can participate in the construction of scaffold that creates *in vivo*-like conditions. In this regard, we fabricated a testicular extract ECM-enriched gelatin foam through the gas foaming method that creates a porous and interconnected sponge.
Materials and Methods: After decellularization of ram testicular tissue fragments, DAPI, H & E staining, and quantitative evaluation of the DNA content were done to confirm successful cell removal. Alcian blue, Masson's trichrome, and Orcein staining were used to affirm decellularized ECM quality. A porous hybrid scaffold was prepared by combining different

concentrations of testis ECM extract and gelatin through the gas foaming process. The assessment of the mechanical, morphological, and biological properties of hybrid scaffolds was performed.
Results: Histological and DNA content analysis showed successful steady maintenance of ECM components as well as effective cellular removal. Besides, the results presented that the scaffolds with 250 -310 µm pore size, appropriate interconnectivity, and proper mechanical properties were manufactured through the gas foaming technique. The significant increase in cell penetration index, high cell attachment as well as low cell toxicity for testicular cells were visualized in the 3D hybrid scaffold with 5% testicular ECM compared to other groups ($P<0.0001$).
Conclusion: Our data suggest testis ECM-derived 3D scaffolds fabricated by the gas foaming method can provide a novel substrate for the assessment of *in vitro* spermatogenesis and reproductive biology applications.
Keywords: Decellularized Testicular Extracellular Matrix, Gas Foaming Method, Male Infertility

P-73: Isolation and Characterization of Extracellular Vesicles Derived From Human Theca Cells Differentiated from Human Theca Stem Cells

Mortazavi Nasiri SM[1*], Dalman A[2], Shekari F[3]

1. Developmental Biology, University of Science and Culture, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research , Tehran, Iran
3. Department Stem Cells and Developmental Biology, Cell Science Research, Tehran, Iran
Email: azamdalman@yahoo.co.uk

Background: Theca cells are a part of the follicle that, despite their vital role in follicle maturation, few studies have been done on them. Theca cells affect the function of other follicle components through the factors they released. These factors are transferred to surrounding cells through various ways, one of the most important of which are extracellular vesicles (EV). Theca cell-derived EVs are important for *in vitro* follicular development studies. In this study, we intend to isolate and characterize EVs derived from human Theca cells differentiated from human Theca progenitor cells.
Materials and Methods: Human Theca stem cells were isolated from the theca layer of small antral follicles (3-5 mm in size). Isolated hTSCs were expanded and cultured in a differentiation medium for 11 days. After differentiation into human Theca cells, their supernatant or condition medium was removed and after centrifugation, their EVs were isolated and confirmed their identity by SDS_page (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis), characterization of specific markers (CD81, CD63, CD9, TSG101) of EVs by western blotting, and checking their size distribution by DLS (Dynamic Light Scattering). And then we check the presence of proteins derived from theca cells (TGF_β, BMP4) in EVs by western blotting.
Results: Our results show that the EVs were isolated and three of the most important markers of EVs were well expressed. Their size was between 100 and 400 nm, and the SEM images confirmed that they were spherical. Moreover, TGF_β and BMP4 were expressed in theca cells EVs.
Conclusion: EVs derived from theca cells were successfully isolated and characterized. Therefore, we plan to investigate the effect of these EVs on *in vitro* development of granulosa cells in the future.
Keywords: Exosomes, Extracellular Vesicle, Ovarian Follicle, Theca Cell

P-74: Coenzyme Q10 Treatment Ameliorating Impacts on Testicular Detorsion/Reperfusion-Induced Apoptosis in Rats

Navidian M*, Madani J, Gharibeh N

Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Email: javadmadani3380@gmail.com

Background: During the last decades, numerous antioxidants have been utilized to inhibit testicular torsion/reperfusion (TT/TR) injuries. Coenzyme Q10 (CoQ10) is a well-known antioxidant agent that has been used to improve infertility. This study aims to investigate CoQ10 effects on experimental TT/TD.
Materials and Methods: Thirty-two male mature Wistar rats were randomly divided into sham and experimental groups (n=8 rats/group). TT was induced in twenty-four rats (2 hours), of which eight rats were considered as the TT group, and TD was performed on sixteen rats (2 hours). Eight animals received normal saline (TD/NS group) and others received CoQ10 (10mg/kg; TD/SCoQ10 group) injection 30 minutes before TD surgery. Testicles were dissected for histopathological (Johnsen's score) and immunohistochemical (Bcl-2, Bax, Caspase3) analysis.
Results: The number of Bax and Caspase-3 positive cells per mm^2 of tissue was up-regulated in the TT and TD/NS groups versus the Sham group, while the number of Bcl-2 positive cells was diminished in the TT and TD/NS groups compared to the Sham group. Moreover, spermatogenesis was arrested, and Johnsen's score was reduced in the TT and TD/NS groups compared to the Sham group. CoQ10 treatment increased the Johnsen score and the number of Bcl-2 positive cells while reducing the number of Bax and Caspase-3 positive cells in the TD/CoQ10 group compared to the TT and TD/NS groups.
Conclusion: Based on the current study's findings, it can be concluded that CoQ10 injection (10mg/kg) 30 minutes before TD can positively improve the germ cell survival rate in the testis, leading to an improved Johnsen score, demonstrating a possible spermatogenesis re-initiation process.
Keywords: Apoptosis, Coenzyme Q10, Rat, Testicular detorsion, Testicular Torsion

P-75: Analyzing The Morphometric Characteristics of Competent Oocytes for Assisted Reproductive Technologies

Parham A[1, 2*], Vajed Ebrahimi MT[1], Attaranzadeh A[3], Golzarian MR[4]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Stem Cell Biology and Regenerative Medicine Research Group, Research Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Milad Center for Infertility Treatment, Mashhad, Iran
4. Department of Biosystems Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
Email: parham@um.ac.ir

Background: The selection of oocytes in assisted reproductive technology (ART) primarily relies on morphological characteristics, however, the current grading and screening criteria still contain subjective elements. Here, we assessed the morphometric (instead of morphological) characteristics of oocytes which were selected as the most competent candidates for intracytoplasmic sperm injection (ICSI) procedure.
Materials and Methods: Here, we assessed the morphometric (instead of morphological) characteristics of oocytes which were selected as the most competent candidates for intracytoplasmic sperm injection (ICSI) procedure. 30 women, with a mean age of 31.9 years, underwent controlled ovarian stimulation for ICSI. A total of 115 oocytes were collected. The oocyte samples were categorized as low and high quality by an embryologist and subjected to imaging. Then, various morphometric characteristics were analyzed using ImageJ software (Version 1.54b). The analysis included measurements such as oocyte diameter and area, ooplasm area and diameter, the range of perivitelline space width (minimum and maximum), zona pellucida thickness, dimensions (diameter and area) of the first polar body, as well as the ratio between the ooplasm area and polar body area.
Results: The results showed that the mean diameters and the area of the ooplasm in competent oocytes, were 116.3 μm and $11.1*10^4$ μm^2, respectively, which were not significantly different from those of low-quality oocytes (112.4 μm and $9.7*10^4$ μm^2). The maximum width of the perivitelline space in competent oocytes was equal to the diameter of the polar body and its location. The ratio of the area of the ooplasm to the polar body area of the competent oocyte was 31.5 ± 5.2, and the quality of the prepared zygotes was better in this range. The average thickness of the zona pellucida was 17.6 μm, with competent oocytes displaying a uniform and thinner zona pellucida compared to low-quality oocytes.
Conclusion: It can be concluded that morphometric characteristics are more accurate than morphological characteristics for oocyte selection in ART centers which can increase the rate of pregnancy.
Keywords: ART, Competent Oocytes, ICSI, Morphometric

P-76: The Evaluation Effect of Nonoliposome with Mitotempo on Sperm Parameters during Cryopreservation in Humans

Piroozmanesh H[1*], Jannatifar R[2], Asa E[2], Verdi A[2], Sahraei S[2]

1. Department of Biology, Arak University, Arak Branch, Arak, Iran
2. Department of Reproductive Biology, Academic Center for Educa;on Culture and Research (ACECR), Academic Center for Education Culture and Research (ACECR), Qom, Iran
Email: hp457@yahoo.com

Background: Cryopreservation of sperm has increasingly become an essential technique that allows sperm to maintain biological function and genetic diversity. However, it is known that the freezing of sperm negatively influences the sperm parameters by producing reactive oxygen species (ROS). MitoTempo has antioxidant activities and can reduce the amount of ROS in the cell, so it may protect sperm from ROS damages. The aim of this study is the evaluation effect of Nonoliposome with MitoTempo on sperm parameters during cryopreservation in humans.
Materials and Methods: In this study, 50 men (random) were selected to refer to infertility treatment Center Qom. semen samples were collected after 2-7 days of sexual abstinence period from patients. Then the sperm parameters and DNA fragmentation index (DFI) were analyzed according to instruments WHO (2010) and following it, each sample was divided into 5 groups(E1-E5). E1: in the control group the cryopreservation sperm without Nonoliposome and MitoTempo and only freezing medium. E2: in this group the cryopreservation sperm with Nonoliposome and MitoTempo (0.5mmol) + freezing medium. E3: In this group the cryopreservation sperm with Nonoliposome and MitoTempo (5mmol) + freezing medium. E4: In this group the cryopreservation sperm with Nonoliposome and MitoTempo (50mmol) + freezing medium. E5: in this group the cryopreservation Sperm with MitoTempo 5 mmol + freezing medium.
Results: The result of this study indicated that sperm parameters and the percentage of DFI significantly increase and decrease in E2, E3, and E4 groups, compared to E1, and E5 groups respectively. In addition, the sperm parameters in E5 group increased compared to E1 group and the percentage of DFI in E5 group decreased compared to E1 group. Our result indicated the sperm parameters in E3 group significantly increased compared to E2, and E4 groups according to our result, the percentage of DFI in E3 group significantly decreased compared to other groups.
Conclusion: MitoTempo was a highly effective antioxidant due to not only the structural features but also the capability to collaborate with lipid bilayers. Consequently, the composition of Mito-Tempo with Nonoliposome leads to a significant function in reducing oxidative destruction and causing increasing quality of sperm parameters.
Keywords: Cryopreservation, DNA Fragmentation, Mitotempo, Sperm

P-77: The Effect of L-Carnitine Oral Solution in Sperm Parameters of High Fat Diet-Received Mice

Roshanfekr Rad M[1*], Razi M[2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Tehran, Iran
2. Department of Comparative Histology & Embryology, Faculty of Veterinary Medicine, Urmia, Iran
Email: Moona.roshanfekr@gmail.com

Background: The purpose of this research was investigating the effect of L-carnitine (2000 mg) oral solution (250 mg/kg) as a cytoprotective agent with antioxidant properties on Sperm parameters of High fat diet-received mice.
Materials and Methods: Immature male mice were randomly divided into three groups as: Control group which received normal diet, High fat diet-received group (HFD) which received high fat diet for 30 weeks, and high fat diet-received group which received high-fat diet for 30 weeks and also received L-carnitine for the last 8 weeks (HFD+LC) of the research period (30 weeks). Body weight changes, testis weight, gonadosomatic index (GSI), adiposity index (AI), sperm count, Viability, chromatin integrity, and testosterone level were measured.
Results: HFD+LC mice showed a remarkable ($P<0.05$) decrease in body weight changes compared to HFD-received group. Testis weight and GSI were significantly ($P<0.05$) increased in the HFD+LC group compared to the HFD and Control groups. AI and sperm count didn't change significantly ($P<0.05$) in experimental groups compared with the control

group. The HFD group showed a significant (P<0.05) increase in viability compared to the control group but HFD+LC mice didn't show any significant (P<0.05) differences versus the control group. Regarding chromatin integrity, the HFD mice exhibited a significant (P<0.05) decrease versus control group. HFD and HFD+LC mice showed a remarkable (P<0.05) increase in testosterone level versus control group.
Conclusion: We conclude from our findings that L-carnitine oral solution as one kind of bodybuilding solution can suitably save the body's male system balance even in high-fat diet-received situations.
Keywords: Body Weight Changes, High Fat Diet, L-Carnitine, Mice, Testosterone

P-78: How Xylitol Makes Different Effects in Sperm Quality Parameters of High Fat Diet-Received Mice; An Experimental Study

Roshanfekr Rad M[1*], Sheibani MT, Razi M[2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Tehran, Iran
2. Department of Comparative Histology & Embryology, Faculty of Veterinary Medicine, Urmia, Iran
Email: Moona.roshanfekr@gmail.com

Background: The aim of this study was investigating the effect of Xylitol as one kind of alcoholic sugar and powerful antioxidant on high-fat diet-received mice.
Materials and Methods: Immature male mice were randomly assigned to 6 groups: the control group which received normal diet, high fat diet-induced obesity resistant group (NH), high fat diet-induced obesity group (H), Xylitol-received group (X), high fat diet-induced obesity which received Xylitol (HX), and high fat diet-induced obesity which received high fat diet at the first 8 weeks and Xylitol for the second 8 weeks (HsX). The period of our research was 16 weeks and all high fat diet-induced groups received high fat diet for 16 weeks except the last group. The body weight changes, testis weight, gonadosomatic index (GSI), adiposity index (AI), the sperm count, viability, and chromatin integrity as well as DNA damage were examined. The serum testosterone, total antioxidant capacity (TAC) of serum and testis, and malondialdehyde (MDA) levels were also measured and compared between groups.
Results: Our results showed that the body weight was significantly (P<0.05) decreased in X, HX, and HsX groups versus HFD and control groups. Testis weight and GSI percentage didn't show any significant (P<0.05) differences in the experimental groups compared with the control group. AI was significantly (P<0.05) increased in the HFD group compared to the control group. Regarding the sperm count, the results showed remarkable decrease in NHFD and HFD groups compared to the control group and we have a significant increase in HX group versus the HFD group. The HX and HsX mice didn't exhibit any significant (P<0.05) changes in sperm viability and chromatin integrity versus the HFD group. All the experimental groups didn't exhibit significant changes in DNA damage compared with the control group. Testosterone level was remarkably (P<0.05) increased in HX mice when compared to the control group. HX mice showed remarkable (P<0.05) increment in TAC level of serum versus HFD mice. HX and HsX mice didn't show any significant changes in TAC level of tissue versus HFD mice. MDA level in HX and HsX mice was remarkably (P<0.05) decreased compared to the HFD group.
Conclusion: Our findings show that despite of positive effect of Xylitol on weight loss and male reproductive system, it shows different aspects of its antioxidant power when goes along with high fat diet consumption.
Keywords: Adiposity Index, Body Weight Changes, High-Fat Diet, Mice, Xylitol

P-79: The Effects of Overcrowding on Mouse Oocyte Quality and DNA Fragmentation

Sabbaghziarani F[*], Rajaei F

Department of Anatomical sciences, Qazvin University of Medical Sciences, Qazvin , Iran
Cellular and Molecular Research Center, Research Institute for Prevention of Non-Communicable Diseases, Qazvin University of Medical Sciences , Qazvin , Iran
Email: farzadraj@yahoo.co.uk

Background: Repeated or prolonged stress can have deleterious effects, such as suppression of reproduction and higher rates of programmed cell death. The present study focuses on the effects of overcrowding on mouse oocyte quality and DNA fragmentation.
Materials and Methods: Female mice were divided into control groups (5 mice per cage), low-stress groups (10 mice per cage), and high-stress groups (15 mice per cage) and kept for one month. Mice in all groups were superovulated with Pregnant Mare Serum Gonadotropin (PMSG) and human chorionic gonadotropin (hCG) and sacrificed by cervical dislocation 15 hours after hCG injection. Oocytes were collected from the fallopian tubes of mice. Grade I and IV oocytes of mice. Grade I and IV oocytes were selected, their number and size determined and stained by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method to detect DNA fragmentation. Data were compared using the statistical method of analysis of variance (ANOVA), and a $p \leq 0.05$ was considered significant.
Results: The data showed that the number of grades I and the size of grade I and IV oocytes decreased significantly in the high-stress group compared to the control group. The rate of DNA fragmentation of grade I and IV oocytes was significantly increased in the low and high-stress groups compared to the control group (all $P \leq 0.05$).
Conclusion: These results suggest that crowding stress may affect DNA fragmentation through changes in oocyte quality.
Keywords: Crowding Stress DNA Fragmentation, Mouse, Oocyte

P-80: Pentoxifylline Attenuates Doxorubicin-Induced Testicular Toxicity in Mice by Reducing Oxidative Stress

Shafiei Z[*], Mahmoodi M

Department of Biology, Arak university, Arak, Iran
Email: Zshafie373@gmail.com

Background: Doxorubicin (DOX) is a widely used chemotherapeutic agent causing testicular toxicity and might lead to male infertility. The present study aims to investigate the protective effects of pentoxifylline (PTX) as a potent antioxidant on DOX-induced testicular injury.
Materials and Methods: 24 adult male Naval Medical Research Institute (NMRI) mice were randomly divided into four

groups: Control, DOX (2 mg/kg/weekly), PTX (100 mg/kg/daily) and PTX+ DOX. After 35 days of intraperitoneal treatment, the left testis was removed and after fixation, tissue processing and staining by hematoxylin-eosin (H&E) method, was examined by stereology technique. The serum concentration of testosterone hormone, malondialdehyde (MDA) and the total antioxidant capacity (TAC) were assessed. The results were analyzed using one-way ANOVA and Tukey's test.
Results: The results showed a significant decrease in body and testicular weight, total volume of testes, volume and length of seminiferous tubules and also diameter and height of the germinal epithelium, the number of germ cells, spermatogenesis indexes, testosterone and TAC level in the DOX treatment group compared to the control group ($P<0.001$). Significant increase was observed in the volume of interstitial tissue and MDA level in the DOX group compared to the control group ($P<0.001$). In co-treatment group, PTX significantly reduced the toxic effects of DOX and improved the above parameters compared to the DOX group.
Conclusion: The results showed that PTX as an antioxidant is beneficial in reducing the toxic effects of DOX in testis by enhancing antioxidant activity and decreasing MDA and oxidative stress.
Keywords: Doxorubicin, Oxidative Stress, Pentoxifylline, Spermatogenesis, Testis

P-81: A Comparison Between Wharton's Jelly/Alginate and Collagen/Alginate for Human Ovarian Tissue Bioengineering

Tajbakhsh F[1, 2*], Tavana S[2], Kazemi Ashtiani M[3], Moini A[4, 6, 5], Fathi R[2]

1. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. , Breast Disease Research Center (BDRC), Tehran University of Medical Sciences, Tehran, Iran
6. Department of Gynecology and Obstetrics, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: rfathi79@royaninstitute.org

Background: Artificial ovary is approximately a newfound idea to help pre-pubertal girls and women who cannot delay their disease treatment program. The bioengineered ovary is designed for follicle growth supporting, hormonal cycle return, as well as preventing malignant cell recurrence. In this case, choosing the appropriate matrix has a key role in this purpose. Wharton's jelly and Collagen are two important materials in the female reproductive system. So, this study aims to compare these two biomaterials for human *in vitro* follicular 3D xenotransplantation.
Materials and Methods: Two experimental groups including Wj/Alg and Coll/Alg containing 40 human isolated follicles were xenotransplanted to the right side of the peritoneal pocket of 6-8 weeks ovariectomized NMRI mice for 1 week. Histological analysis and hormonal assessments were employed for the investigation.
Results: Most of the follicles were missed after xenotransplantation due to a lack of enough vascularization. But Hematoxylin and Eosin staining confirms the presence of human xenotransplanted follicles in both groups. On the other side, there are no significant differences between hormonal assessments in both groups.
Conclusion: It seems that Wj/Alg like Coll/Alg can be the suitable matrix to support human ovarian follicles xenotransplantation as a human artificial ovary. However, more experiment needs to improve this system.
Keywords: Artificial Ovary, Collagen, Human Ovarian Follicle, Wharton's Jelly Hydrogel, Xenotransplantation

P-82: The Effect of Microparticle based on Polystyrene on Histomorphometric and Oxidative Stress Factors of Prostate Tissue *In Vivo*

Zangene S*, Morovvati H , Anbara H

Department of Basic Science, University of Tehran, Tehran, Iran
Email: hmorovvati@ut.ac.ir

Background: Microplastics (MP) are tiny pieces of plastic that are widely dispersed in the environment. Studies have been done on the effect of polystyrene microplastic (PS-MPs) on the testes, but the information about the appendages, including the prostate, is still unclear.
Materials and Methods: 36 mature male mice were randomly divided into four groups, each with nine mice. The three groups were gavaged PS-MPs for 42 days at concentrations of 0.01, 0.1, and 1 mg/kg BW. Additionally, by gavage, purified water was given to the control group. After the final treatment, tissue samples were obtained 24 hours later. Mice were used to collect prostate tissue samples, prepare the tissue, and stain the slides with H&E.
Results: PS-MPs administration significantly ($P<0.05$) induced prostate toxicity as evidenced by alteration of serum testosterone, LH, FSH, and PSA. PS-MPs groups showed a significant decrease ($P<0.05$) in cell count and epithelial height of the secretory units in the ventral, anterior, and dorsal lobes of the prostate ($P<0.05$). PS-MPs groups demonstrated a significantly lower percentage of parenchyma in the prostate gland's ventral lobe ($P<0.05$). The up-regulation of MDA and the down-regulation of TAC, SOD, CAT, and GSH are further indications that PS-MPs greatly increased oxidative stress.
Conclusion: Our research revealed that the 42-day delivery of PS-MPs to mice resulted in significant alterations in the prostate's size and tissue composition, which may have an impact on fertility.
Keywords: Histomorphometry, Oxidative Stress, PS-Mps

P-83: Effect of Cyclophosphamide on Ovarian Reserve of Prepubertal Mice

Zatalian N[1, 2*], Gourabi H[2], Afsharian P[2], Dalman A[3]

1. Department of Molecular Cell Biology-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 Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Email: a.dalman@royan-rc.ac.ir

Background: Maintaining the pool of primordial follicles (PFs) is one of the most critical factors in the fertility preservation of girls. Many factors affect this follicular reserve and cause the over-activation of PFs or their apoptosis; Among these factors, chemotherapy drugs such as cyclophosphamide can be mentioned. We aimed at the effect of different doses of cyclophosphamide on prepubertal mice.
Materials and Methods: Four groups of C57BL/6J mice were selected along with their corresponding control group; group-1/2: 28-day-old mice were injected with a single dose of 75mg/kg cyclophosphamide and sacrificed on day 35 (group-1) and day 42 (group-2), group-3/4: 14-day-old mice were injected with three or four doses of 75mg/kg cyclophosphamide once every four days and sacrificed on the 26th and 29th, respectively. Ovaries were removed, and after H&E staining, follicles were counted in both PFs and growing follicles (GFs) and then each group was compared with its control group.
Results: In groups-3/4 compared to the control group, the percentage of PFs significantly decreased ($P<0.05$), and the rate of GFs and atretic follicles (AFs) increased ($P<0.05$). Comparing different groups, the rate of PFs decreased respectively ($P<0.05$) (except between groups-1/2), and the rate of AFs (except between groups-1/2) and GFs increased respectively ($P<0.05$). Generally, reducing the rate of PFs and increasing the rate of GFs was observed with increasing doses of cyclophosphamide, which leads to an excessive decrease in ovarian reserve.
Conclusion: Therefore, it is necessary to preserve ovarian reserves in girls or women who are treated with cyclophosphamide and intend to become pregnant in the future.
Keywords: Ovarian Reserve, Cyclophosphamide, Primordial Follicle, Growing Follicle, Atretic Follicle

Epidemiology and Helaths

P-84: Legislations and Guidelines on Embryo Donation in Iran, The United States of America, England, and Australia: A Qualitative Comparative Study

Iranifard E[1*], Latifnejad Roudsari R[2]

1. Student research committee, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Midwifery, Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Email: rlatifnejad@yahoo.com

Background: Transparent rules and regulations for embryo donation are necessary to maintain the safety, dignity, and rights of donors, recipients, and offspring. The aim of this study was to Compare the laws and guidelines for embryo donation in Iran to those of the United States of America (USA), England, and Australia.
Materials and Methods: To conduct this qualitative comparative study, legal documents and/or guidelines were selected purposefully through a comprehensive data search. Six documents including A legal bill and a regulation document from Iran, a guidance from USA, a code of practice from England, and a guideline as well as a code of practice from Australia, were compared using content Analysis approach.
Results: Three themes were identified including "legislations and guidelines regarding the donors", "legislations and guidelines regarding the recipients" and "legislations and guidelines regarding The donor-conceived offspring". The major differences concerning donors were related to launching a registry and providing counseling services. Regarding recipients, counseling and eligibility criteria for receiving donation treatment were drastically different. The right to know genetic origins, determination of legal parents and well-being of donor-conceived offspring were also vastly different across the countries under study.
Conclusion: Compared to developed countries, the act and executive regulations on how to donate embryos to infertile couples in Iran are faced with major limitations. Therefore, it is necessary to revise and/or update the current law and practical guideline according to the cultural, social, and religious values of Iran to meet the needs of donors, recipients, and offsprings through embryo Donation procedure.
Keywords: Comparative Study Embryo Donation, Guideline, Legislation

Female Infertility

P-85: Effect of Nano-Curcumin on Bcl-2 and Bax Expression in Estradiol Valerate Induced PCOS in Mice

Aaly gharibeh Z[1*], Hosseinchi M[3], Shalizar Jalali A[2, 3]

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
2. Department of Basic Science, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
3. Department of Basic Sciences, Faculty of Veterinery Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
Email: dvm_06@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder and the leading cause of anovulatory infertility. Turmeric rhizome contains a high number of polyphenols (bioflavonoids) called curcuminoids, which comprise curcumin, demethoxycurcumin and methoxycurcumin. Studies on curcumin have demonstrated its benefits due to its antioxidant, anti-inflammatory properties. The aim of this study was to evaluate if the expression of Bax (proapoptotic protein) and Bcl-2 (antiapoptotic protein) in a PCOS model developed in mice by EV (estradiol valerate) administration.
Materials and Methods: 24 adult female mice were divided into four groups of six animals each. Three groups of mice were administered EV (0.2 mg/kg/day, s.c.) for 21 days. Two of these groups received Nano-curcumin (7.5&15 mg/kg/day orally) concurrently. A control group was also included. Bcl-2 and Bax protein concentration was quantified by ELISA kits.
Results: EV treatment caused a significant decrease in the expression levels of Bcl-2 and increase in the expression levels of Bax than those of control. Administration of nano-curcumin, to EV receiving mice, caused a significant increase in the expression levels of Bcl-2 and decrease in the expression levels of Bax than those of EV treatment group.
Conclusion: Nano-curcumin by increase in the expression levels of Bcl-2 and decrease in the expression levels of Bax, inhibits oocyte apoptosis. Thus, Nano-curcumin may be useful in the treatment of PCOS but further clinical trials are required to confirm it.
Keywords: Apoptotic Protein, Mouse, Nano-Curcumin, PCOS

P-86: Protective Effect of Nano-Curcumin on Fertilization Rate in PCOS Mice

Aaly Gharibeh Z[1*], Hosseinchi M[2], Shalizar Jalali A[3]

1. Department of Veterinary Medicne, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
2. Department of Basic Science, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: dvm_06@yahoo.com

Background: By comprising reproductive, endocrine, metabolic and psychological features the cause of PCOS is still unknown. The polycystic ovary syndrome (PCOS) is one of the most common causes of oligo-ovulatory infertility, affecting approximately 4% of reproductive-aged women. PCOS patients frequently have metabolic disturbances which closely resemble the metabolic syndrome. Curcumin is extracted from Curcuma longa and regulates the intracellular signal pathways that can target the intracellular enzymes, genome (DNA) and messengers (RNA). Some studies have been conducted beneficial effects of curcumin on different diseases like diabetes, cancer, Alzheimer's and infertility. The aim of this study was to explore that the hypothesis Nano-curcumin may be protective against estradiol valerate (EV)-induced PCOS through antioxidant-mediated mechanisms.
Materials and Methods: Experiments were performed on four groups each consisting of six mice. EV reproductive toxicity was induced by subcutaneous injection of EV at dose of 0.2 mg/kg body weight daily for 21 days. EV plus Nano-curcumin groups received (7.5&15mg/kg/day, oral). Corresponding control groups were also used.
Results: Administration of EV caused a significant decrease in fertilization rate along with poor blastocyst formation in EV-treated animals than those of control. Administration of Nano-curcumin, to EV receiving mice, markedly attenuated EV-induced embryotoxicity and ameliorated negative changes observed in the above-mentioned parameters.
Conclusion: Findings from this study suggest that Nano-curcumin has a potential repro-protective action against EV-induced embryotoxicity in mice. However, clinical studies are warranted to investigate such an effect in human subjects.
Keywords: Embryo, Mouse, Nano-Curcumin, PCOS

P-87: Nano-curcumin Regulates Testosterone Levels in Estradiol Valerate-Induced Polycystic Ovary Syndrome (PCOS)

Aaly Gharibeh Z[1*], Hosseinchi M[2], Shalizar Jalali A[3]

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, Urmia Branch Islamic Azad University, Urmia, Iran
2. Department of Basic Science, Faculty of Veterinary Medicine, Islamic Azad University, Urmia, Iran
3. Department of Basic Sciences, Faculty of Veterinery Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
Email: dvm_06@yahoo.com

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous condition that affects 6–20% of women, making it the most common endocrine condition in women of reproductive age. Elevated androgens represent a major feature of PCOS because the majority of women suffering from PCOS are hyper androgenic. Hyper androgenic PCOS women display an elevation in serum levels of various androgens, including testosterone. Curcumin is a naturally phenolic compound that very promising bioactivities has been identified or attributed to curcumin, notably antioxidant. The current study was designed to investigate the efficacy of Nano-curcumin in regulate the testosterone levels in PCOS mice.
Materials and Methods: Adult female NMRI mice were assigned into four treatment groups. Three groups of mice received EV (2 mg/kg/day) Subcutaneous Injection. Nano-curcumin was given orally to two of these groups at the dose level of 7.5 and 15 mg/kg body weight per day. Corresponding control groups were also included. Serum levels of the testosterone were measured using ELISA kit.
Results: Serum levels of the testosterone were significantly higher in EV exposed mice than those of control, whilst Nano-curcumin co-administration substantially reduced Serum levels of the testosterone in comparison with EV-only treated group.
Conclusion: Data from the current study suggest that antioxidant activity of Nano-curcumin could increase testosterone level in PCOS mice so it could restore fertility. Therefore, this study confirms remedial effects of Nano-curcumin on PCOS mice infertility.
Keywords: Estradiol Valerate, Nano-Curcumin, PCOS, Testostrone,

P-88: Comparison of The Successful Fertility in Fresh and Frozen Embryo Transfer

Adib-Rad H[*], Basirat Z , Esmailzadeh S , Jorsaraei S Gh A, Ghofrani F , Pasha H

Infertility and Health Reproductive Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
Email: AdibRad2015@yahoo.com

Background: The use of assisted reproductive technology is increasing in the world. Assisted reproductive technology (ART) range, efficacy and safety is vary noticeable among countries. There is an increase in the usage of ICSI, single embryo transfer and frozen embryo transfer. Frozen embryo transfer has no negative effect on the successful fertility compared with fresh embryo transfer.
Materials and Methods: In this retrospective cross sectional study check out the reproductive success of 1014 cycles after ET,588 of which have resulted from frozen-thawed ET and 426 from fresh ET.
Results: The Fertility Rate on the diagnosis tests by type of transferred embryo showed that biochemical pregnancy rate was 23% in fresh ET group versus 18.8% in FET group (OR 1.301; 95% CI .955-1.774) . There was no significant difference between the two groups.
Conclusion: The ART methods are costly, need a notable requirement of time and energy for infertile couples. Therefore, detection out of the affecting factor is a important method to correct their chances of understanding.
Keywords: Fresh Embryo Transfer, Frozen Embryo Transfer, Successful Fertility

P-89: Diet Diversity Score Is Related with Poor Reproductive Health and Pregnancy Outcome

Ahmadi P[*], Abbasi B, Bayat N

Department of Nutrition, Electronic Health and Statistics Surveillance Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: abbasi.b@srbiau.ac.ir

Background: Life style and diet may be related to reproductive cycle. It is suggested that a dietary index called Dietary Diversity Score and defined as the number of different food groups or items consumed in a period of time, might be associated with various outcomes of reproduction. The purpose of this study was to summarize the findings of the relation of dietary diversity score and complications related to reproductive health and pregnancy.
Materials and Methods: A literature search in major databases such as Web of Science, PubMed, Google Scholar, Scopus, and Scientific Information Database was conducted until May 2023. This was done in conjunction with a search in Elsevier and Springer Link databases, resulting in the inclusion of relevant articles in this review.
Results: Our research was conducted based on fifteen articles from 2012-2023 which all contained a link between dietary diversity and reproductive issues. Eight studies were about the association of dietary diversity and pregnancy outcome like neonate's birth weight and gestational weight. The remaining seven studies, investigated reproduction health and dietary diversity relevance.
Conclusion: Based on our findings a higher Dietary Diversity Score was associated with lower risk of reproductive health disorders such as polycystic ovary syndrome, maternal anemia and bone status. A diversified diet may reduce risk of some birth outcomes including low birth weight infant, Apgar score and congenital heart defect. Meanwhile, results showed that DDS was not related with gestational hypertension and diabetes and weight gain.
Keywords: Birth Weight, Dietary Diversity, Pregnancy Complications, Reproductive Health

P-90: Investigating The Effect of Stress During Pregnancy on The Estrogene, Progesterone, FSH/LH Blood Levels, Lipid Peroxidation, DNA Damage, and Oocyte Number in First Generation Bicuculline -Treated Female Rats

Ahmadzadeh R[1*], Roshan-Milani Sh[2], Saboory E[3]

1. Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Physiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
3. Department of Addiction Studies, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
Email: raminahmadzadeh62biology@gmail.com

Background: Secreted hormons from ovary, pituitary glands and suprarenal gland may affect the follicogenesis and the number of mature oocyte. Stress during pregnancy causes unusual neuronal connections in the fetal development which leads to seizure potentiation and alteration in activity of endocrine glands.
Materials and Methods: Pregnant rats (180-220 g) were divided in two groups (n=6 each): control and stress. In the stress group, the rats were kept immobile on the 15th day of pregnancy by restrainer twice a day, for one hour, and up to 3 consecutive days. On the 25th day after birth, bicuculline (150 mg/kg.s.c) was injected to the female pups of both groups to induce epileptic behaviors. On 70th day after birth, the subjects were anesthetized and studied in terms of estrogen, progesterone, FSH/LH blood levels, and oocyte number.
Results: The mean of estrogen, progesterone, FSH/LH blood levels, oocyte number in the epileptic stress group decreased significantly compared to the control group. While, the mean of Corticosterone and lipid peroxidation were significantly increased in the epileptic stress group compared to the epileptic control group.
Conclusion: It seems that the stress during the fetal period can affect the hypothalamus-pituitary axis by inhibiting the release of GnRH hormone and the synthesis of gonadotropins from the pituitary by intensifying epileptic behaviors and increasing blood corticosterone levels.
Keywords: Corticosterone, Estrogen and Progesterone Hormones, Gestational Restraint Stress, Lipid Peroxidation

P-91: The Effect of Acupuncture during Ovulation Stimulation on IVF/ICSI Cycle Outcome in Patients with Poor Ovarian Response: A Randomized Clinical Trial

Arabipoor A[1*], Salimi T[1], Ashrafi M[1], Yahyaei A[1], Mohammadi M[2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: arezoo.arabipoor@gmail.com

Background: To determine the effect of acupuncture during the controlled ovarian stimulation (COS) cycle in women with poor ovarian response (POR).
Materials and Methods: This randomized clinical trial was conducted at Royan Institute from March 2020 to January 2022. The patients under 35 years old with a history of POR (less than 4 retrieved oocytes) or abnormal ovarian reserve (POSEIDON group I or III) were included. The COS was performed by standard agonist stop protocol in all the patients. The eligible 43 patients were assigned into two groups randomly by permuted block randomization method. In the experimental group, acupuncture sessions were carried out twice per week in the month prior the onseting of COS and then once per week until the puncture day (totally 8 sessions) based on the WHO Standard Acupuncture Point Locations. In the control group, no intervention was done. The primary outcomes were the number and quality of retrieved oocytes.
Results: Finally, 16 patients in the acupuncture group and 19 patients in the control group performed all the research process completely. The means number of retrieved and MII oocytes in the acupuncture group was higher than the control group (6.36 ± 6.15 vs. 4.62 ± 4.58 and 5.36 ± 4.98 vs. 3.68 ± 3.0, respectively); however, no significant difference was found (P=0.64 and P=0.29).
Conclusion: Forasmuch as the number and quality of the retrieved oocytes after acupuncture was clinically more acceptable and no side effects were observed, therefore, researchers suggest that acupuncture at least 2 months before IVF for POR patients.
Keywords: Acupuncture, Assisted Reproductive Technology, Poor Ovarian Response, Number of Retrieved oocytes

P-92: Evaluation The Effects of Mobile Phone Electromagnetic Waves (900-950 MHZ) on Reproductive Hormones in Female Rats

Asgharyzadeh F[1*], Sadr Forati F[2], Khorsandi N

1. Department of Clinical Sciences, Tehran University, Tehran, Iran
2. Department of Clinical Sciences, Research Sciences, Tehran, Iran
Email: asgharyzadeh_fahimeh_1395@yahoo.com

Background: The widespread uses of tools that generate electromagnetic waves (EMW), especially mobile phones caused scientists investigate the harmful effects of mobile phone waves, these days.
Materials and Methods: Rats were divided in to 2 groups (n=12/each): group 1: without EMW(control group) , group 2 (experimental group): rats were exposed to mobile phone EMW, 4 hr a day for 2 month. After 2 month, rats were sedated with chloroform and blood sampling was done from the heart and serum samples were separated. The concentration of estrogen and progesterone , FSH and LH hormones was measured by ELISA method.
Results: The results showed, estrogen and progesterone levels, significantly decreased in group 2, ($P<0.05$). follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in group 2 were significantly increased, ($P<0.05$) compared to group 1.
Conclusion: Considering the results, it can be concluded that EMW may damage the follicular cell structure by inducing oxidative stress and disorder in the secretion of sex hormones affect the state of reproduction.
Keywords: Electromagnetic Waves, Female Reproductive Hormones, Mobile Phone, Rat

P-93: Genetic and Epigenetic Changes of HOXA10 in Endometrial Tissue of Hydrosalpinx patients Undergoing Laparoscopic Salpingectomy

Baniasad Azad N[1, 2], Zaferani F[2, 3], Ghaffari F[4], Shahhoseini M[2, 6, 7]**

1. Department of Molecular Cell Biology-Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan, Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
6. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
7. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: shahhoseini244@gmail.com

Background: Hydrosalpinx is a blocked fallopian tube often caused by infection, scar tissue or endometriosis, which is usually associated with adverse effect on endometrial receptivity by alteration of key receptivity marker genes such as HOXA10. Previous studies demonstrated that hydrosalpinx is associated with detrimental effect on *in vitro* fertilization (IVF) success rates and salpingectomy improves subsequent pregnancy and live birth rates.
Materials and Methods: Endometrial samples were obtained from 10 patients in reproductive age (20-40 years) with moderate to severe hydrosalpinx proven by hysterosalpingography or laparoscopy. The patients underwent laparoscopic salpingectomy from January 2021 to January 2022. All women had normal hormonal profile, BMI (18–28 kg/m^2) and normal menstrual cycles. Ten healthy fertile age-matched women with a history of successful pregnancy considered as control group. Mid–luteal-phase endometrial pipelle biopsy performed at the time of surgery and second mid–luteal-phase endometrial sampling was obtained in forth-post treatment cycle. Informed consent was obtained from all participants according local ethical approval. Real-time PCR technique was used to evaluate mRNA expression of HOXA10 quantitatively then data were analyzed based on $2^{-\Delta\Delta CT}$ to estimate the relative fold change value. Also, incorporation of H3K9ac/me2 histone marks (as gene activating / repressive epigenetic marks) was evaluated by chromatin immunoprecipitation (ChIP) coupled with real-time PCR. One way ANOVA was used for data analysis. P value less than 0.05 was considered statistically significant.
Results: Expression level of HOXA10 gene revealed significant increase (about 7 folds) in endometrial samples of hydrosalpinx patients after salpingectomy ($P=0.03$), while there was no significant change in expression levels of this gene in endometrial tissues after salpingectomy in comparison to normal endometrium ($P=0.27$). Incorporated levels of mentioned epigenetic marks into promoter of HOXA10 was in alignment with gene expression changes of this gene: high levels of acetylation parallel to low incorporation of methylation of H3K9 histone in patients after salpingectomy.
Conclusion: Our results imply importance of considering of genetic/epigenetic alterations of endometrium (including HOX genes) regarding to hydrosalpinx and salpingectomic surgery, to reach better insight into molecular mechanisms leads to successful ATR.
Keywords: Endometrial Receptivity, Histone Modification, HOXA10 Gene Expression, Hydrosalpinx, Salpingectomy

P-94: Life Style and Reproductive Risk Factors in Breast Cancer

Bayat H[1, 2*], Nazarpour S[3], Anbiaee R[4]

1. Department of Anatomical Sciences and Cognitive Neuroscience, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. Department of Midwifery, Varamin-Pishva Branch, Islamic Azad University, Tehran, Iran
3. Department of Radiation Oncology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: snazarpour@gmail.com

Background: Breast cancer is one of the most common worldwide cancers and causes of death. Various risk factors influence the risk of breast cancer. In this study, we investigated breast cancer risk factors in Iranian women. The aim of this study was to investigate reproductive risk factors for breast cancer.
Materials and Methods: This case-control study was conducted between September 2019 and November 2022 in two hospitals in Tehran. In this study, 250 women with breast cancer were compared with 250 women without breast cancer as a

control group. Data collection was done through checklists with questions about reproductive variables. Logistic regression was used and the level of statistical significance was set at P<0.05 for all the tests

Results: Our results showed stress [OR: 1.77 (95% CI:1.51-2.09) (P<0.001)], using high-fat foods [OR: 0.39 (95% CI:0.29-0.53) (P<0.001)], lower education level [OR:0.34 (95% CI:0.28-0.42) (P<0.001)], abortion history [OR:2.54 (95% CI:1.69-3.86) (P<0.001)], more children number [OR: 1.04 (95% CI:2.19-3.74) (P<0.001)] and prolonged breastfeeding [OR:2.01 (95% CI:1.63-2.49) (P<0.001)] increase the chance of breast cancer. On the other hand, regular menstrual cycles get less breast cancer [OR: 0.61 (95% CI: 0.39-0.95) (P<0.03)].

Conclusion: Our study demonstrated that lifestyles such as stress and mental pressure, nutrition and education, and reproductive factors are associated with breast cancer risk.

Keywords: Breast Cancer, Life Style, Malignancy, Reproductive, Risk Factors

P-95: Relation of Follicular Fluid Soluble Receptor for Advanced Glycation End-Products (sRAGE) Concentration on Ovarian Reserve in PCOS and Non-PCOS Women Referring to IVF Center

Emami N[1*], Moini A[2, 3], Maleki-Hajiagha A[4]

1. Department of Gynecology and Obstetrics, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Gynecology and Obstetrics, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research
Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: neda.emami58@gmail.com

Background: The reproductive dysfunctions of polycystic ovary syndrome (PCOS) are significantly influenced by the dietary advanced glycation end products (AGEs). The interplay between AGEs and their receptor, known as the receptor for advanced glycation end products (RAGE), is closely associated with abnormal ovarian follicular growth. RAGE has a soluble form, (sRAGE), which might exert a protective role on the follicular environment and affect AMH concentration.

Materials and Methods: A total of forty-three women of reproductive age participated in this case- control study, with twenty-three non-PCOS women assigned to the control group and seventeen patients diagnosed with PCOS allocated to the case group. Prior to the IVF procedure, fluid samples were collected from the first large aspirated follicle. The levels of FF sRAGEs and serum AMH were recorded through the use of commercially available ELISA kits. Our objective is to investigating the relationship between sRAGE levels in follicular fluid (FF) and serum AMH levels in PCOS and non-PCOS women.

Results: Correlation analysis, without age matching, revealed a statistically considerable and positive association between FF sRAG and serum AMH concentration in PCOS women. (P=0.05, r=0.0596). Moreover, in PCOS women aged 40 years or older, as well as those younger than 30 years, correlation analysis demonstrated a significant and positive relationship between FF sRAGE and serum AMH levels (P=0.01, r=1).

Conclusion: The association between sRAGE and AMH in women with PCOS is primarily affected by their age, whereas non-PCOS women showed no relationship.

Keywords: Advanced Glycation End Products (AEGs), Intra Follicular Fluid, IVF, PCOS, Soluble Receptor

P-96: Endometriosis Recurrence in Infertile Women Treated by Assisted Reproductive Technology and Surgery

Farzizadeh N*, Shahbazzadegan S

Department of Nursing and Midwifery, Ardabil University of Medical Sciences, Ardabil, Iran
Email: nedafarziezadeh1@gmail.com

Background: Endometriosis is a benign gynecologic disease which up to 30-50% of infertile women characterized by it. Infertility treatments such as laparoscopic surgery and assisted reproductive technology (ART) increase the chance of conception in women with endometriosis. It is still not clear which treatment is more likely to result in endometriosis recurrence.

Materials and Methods: This abstract is prepared by searching the keywords of endometriosis recurrence, infertility, and assisted reproductive technologies in Google Scholar and PubMed databases.

Results: Based on moderate quality evidence, *in vitro* fertilization (IVF) does not increase the risk of endometriosis recurrence, albeit low quality evidence indicates intrauterine insemination (IUI) may increase the risk of endometriosis recurrence. Furthermore, the risk of endometriosis recurrence is not associated with the number of IVF cycles and the responsiveness to ovarian hyperstimulation (OH). Additionally, the cumulative endometriosis recurrence rate is lower after OH for IVF than after lower-dose ovarian stimulation for IUI, suggesting that temporary exposure to high estradiol levels during OH for IVF is not a major risk factor for endometriosis recurrence in women treated with ART. Moreover, there is no significant difference between the rate of endometriosis recurrence in infertile women treated by surgery and ART.

Conclusion: It seems that the risk of endometriosis recurrence is not soared by ART (especially IVF) or surgery and there is no necessary to do prophylactic surgery before ART treatment to prevent endometriosis recurrence.

Keywords: Assisted Reproductive Technologies, Endometriosis Recurrence, Infertility

P-97: Increased Expression of HOX C12 and HOX C13 Genesin Plasma of Women with Endometriosis

Favaedi R[1*], Hayaei Tehrani RS[2], Karbalaee N[1, 3], Mikaeeli G[1, 4], Bakhtiari Sh[1, 3], Ghaffari F[5], Moini A[5], Shekari F[2, 6], Esfandiari F[2], Shahhoseini M[1, 8, 7]

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 Molecular Genetics, Faculty of Basic Sciences and advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
4. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
5. Department of Endocrinology and Female Infertility, Reproduc-

tive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
6. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
7. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
8. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: m.shahhoseini@royaninstitute.org

Background: Dynamically expressed HOX genes in endometrium, are necessary for endometrial growth, differentiation, and implantation. Our previous study, showed differences in HOX family expression in the endometriotic tissues of patients compared to normal endometrium. But finding less or non-invasive methods is always a priority. Extracellular Vesicles (EVs) which carry macromolecules of their origin cell, are present in body fluids and are majeure contributors through progression of disease so, they are good candidate for studying diseases by minimally invasive methods. In this study our main goal was to study expression of HOX C12 and HOX C13 in endometriosis using EVs in blood circulation.
Materials and Methods: After obtaining written consent according to the local ethical approval, peripheral blood samples were collected from endometriosis patients (stages III and IV) and women without endometriosis (male factor infertility) (n=30, in each group). EVs separated from plasma by ultracentrifuge then characterized by morphology, DSL and western blot (CD81, CD9, TSG101). Then RNA of plasma extracted using Plasma/ serum circulating and exosomal RNA purification kit. The expression of HOX C12 and HOX C13 evaluated quantitatively by real-time PCR.
Results: Results showed significant higher expression of studied genes in plasma of endometriosis group compared to control group, parallel to previous study comparing the tissues of patients and controls.
Conclusion: Current study confirmed the similarity of results of gene expression comparison in plasma and tissue of endometriosis patient vs. healthy women, also implies HOX C12 and HOX C13 genes can be potential biomarkers for the non-invasive diagnosis of endometriosis.
Keywords: Endometriosis, Extracellular Vesicles, Gene Expression, HOX C12, HOX C13

P-98: Controlled Ovarian Stimulation in Cancer Patients Under 18 Years Old

Ghaffari F*, Yahyaei A, Moridi M

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

Background: Fertility preservation for adolescent pubescent girls is a concern of the healthcare system and parents. Oocyte cryopreservation is regarded as a standard medical intervention for patients with a minimum age of 18 years. Evidence suggests that mature oocyte cryopreservation is possible for adolescent pubescent girls, although, ovarian stimulation for these patients remains a challenge.
Materials and Methods: This case series is the first report regarding ovarian stimulation with oocyte cryopreservation in younger than 18 years cancerous girls, who refer to ROYAN institute, Tehran, Iran, prior to the start of the treatment of cancer (November 2015 to February 2021).
Results: The oocyte cryopreservation was carried out in the 7 patients (five patients with Hodgkin lymphoma, one patient with Ewing sarcoma, and one patient with osteogenic tumor), the embryo cryopreservation in one patient with dysgerminoma, and the oocyte and embryo cryopreservation in one patient with germ cell tumor. No oocytes were retrieved after ovarian stimulation in the patient with medulloblastoma. For one of the patients with Hodgkin lymphoma, half of the tissues of one ovary were cryopreserved prior to ovarian stimulation.
Conclusion: Oocyte cryopreservation is a feasible option of fertility preservation in the adolescent's patients with cancer. However, only if reported acceptable fertilization rates, as well as the successful cases of live birth from oocyte cryopreservation at the ages under 18, this option of preserving fertility can be applied to this age range.
Keywords: Adolescent Pubescent Girls, Cancer Patients, Oncofertility, Fertility Preservation, Controlled Ovarian Stimulation,

P-99: Descriptive Study on Premature Ovarian Insufficiency Patients Referring to Royan Institute

Ghaheri A[1*], Amini Z[2], Amirchaghmaghi E[3], Mirnattaj F[3], Almadani SN[4], Zamanian MR[4], Mashayekhi M[3], Hosseini R[1]

1. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Biostatistics Faculty of Public Health, Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
4. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: azadeh.ghaheri@gmail.com

Background: Premature ovarian insufficiency (POI) is the loss of normal ovarian function before the age of 40 years. POI affects almost 1% of women under 40 years old and almost 0.1% of women under 30 years old. The pathophysiological development of POI patients remains unknown in most cases and needs to be studied. As this condition has life-changing physiological and psychological consequence in young women of reproductive age.
Materials and Methods: This descriptive study aimed to investigate demographic, genetics and clinical information on all premature ovarian insufficiency patients referring to Royan institute since June 2018.
Results: Total number of patients admitted was 337. Their ages ranged from 15 to 47 years (median=35) and almost 61% of these patients were overweight. The median for the menarche age was 13 (Q1=12, Q3=14) and for menopause was 28 (Q1=22, Q3=34). Among the recruited patients 16% had primary amenorrhea and 80.7% had secondary amenorrhea. Hot flashes was reported to be the most common symptom (56.7%) before menopause among these patients. The median for FSH level was 59.7 (interquartile range=51.3). Among these patients 88.55% had normal karyotype.
Conclusion: The results of this study may help to improve our understanding of the etiology, diagnosis and optimal interven-

tion strategies for this condition.
Keywords: Descriptive Study, Premature Ovarian Insufficiency (POI), Royan Institute

P-100: How Does Female Age Influence Outcomes in Different Stages of Intra-Cytoplasmic Sperm Injection (ICSI) procedure Differently?

Ghaheri A[1*], Ramezanali F[2], Rasekhi[4], Omani Samani R[3], Hajizadeh E[4]

1. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Biostatistics, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran
Email: azadeh.ghahei@gmail.com

Background: Women undergoing *in vitro* fertilization (IVF) should go successfully through multiple points during the treatment to achieve live births; previous studies however have often focused on a single point of the procedure as an outcome. While in this study we have considered multiple outcomes. On the other hand, when multiple cycles per couple are analyzed clustering arises; each couple contributes a cluster and each of their cycles is considered a cluster member. Due to underlying biological similarities cycle outcomes are likely to be correlated within a cluster and it is important to consider this correlation in the modeling. In this study we have modeled multiple outcomes during ICSI cycles considering multiple cycles' correlations to explain how each covariate predicts the stage- (outcome-) specific failure probability.
Materials and Methods: In this historical cohort study Clustered-Weighted Generalized Estimating Equations (CWGEE) was used to model the probability of failure at each stage in an ICSI procedure. 1. Blighted ovum, 2. Spontaneous abortion before 12 weeks of pregnancy, 3. Spontaneous abortion before 20 weeks of pregnancy, 4. Delivery to explain how each covariate predicts the stage- (outcome-) specific failure probability. CWGEE accounts for both the correlation among multiple cycles potentially experienced by each couple and the informative cluster size (ICS). Data from 3676 intra-cytoplasmic sperm injection (ICSI) cycles were analyzed. Four main outcomes were modeled simultaneously 1. Blighted ovum, 2. Spontaneous abortion before 12 weeks of pregnancy, 3. Spontaneous abortion before 20 weeks of pregnancy, and 4. Delivery.
Results: The results indicated that while those women over 35 had significantly higher odds of spontaneous abortion before week 12 than those under 35, this difference was not significant among age categories over 35. Allowing the effect of age categories to vary over the failure types in the model indicated that although women over 37 and over 35 (compared to those under 35) did significantly poorer in terminating in blighted ovum and spontaneous abortion before week 12 respectively, but from then on, after passing the first two stages successfully, these women could continue as well as those under 35.
Conclusion: Although female ageing influences the chance of success in fertility treatments, but it is important to determine how it is influencing different stages of treatment procedure.
Keywords: Female Age , Intra-Cytoplasmic Sperm Injection , Multiple Outcome

P-101: Frequency of Peripheral T Cells in Patients with Repeated Implantation Failure / Recurrent Spontaneous Abortion

Habibi S[1*], Amirchaghmaghi E[1], Mostafaei P[1], Sodeifi N[2], Vesali S[3], Ramezanali F[1]

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. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: faribaramazanali@yahoo.com

Background: One of the most important reasons of infertility and human reproductive failure is related to uncontrolled immunological response of maternal immune system to early embryo, so the immune system imbalance during implantation or pregnancy may lead to implantation failure or miscarriage. This study evaluated the frequency of T cells in peripheral blood of recurrent spontaneous abortion (RSA) and/or repeated implantation of failure (RIF) patients.
Materials and Methods: In this study the frequency of T lymphocyte cells in peripheral blood of women with RIF and RSA were studied by flowcytometry. Clinical characteristics and results of immune test were collected from 217 infertile women with RIF, 118 women with RSA and 10 infertile women with a history of both RSA and RIF. Also, 10 fertile women with no history of infertility and miscarriage were enrolled in this study.
Results: The results showed that the frequency of CD3 + cells in the peripheral blood of women with RIF and RSA were close to each other and slightly were lower from the control group. Also, this decrease was significant in the RIF + RSA group compared to the control group. The frequency of CD8 + cells in the peripheral blood of women with RIF and RSA was close to each other and slightly increased to the control group. Also, this increase was significant in the RIF + RSA group compared to the control group. In addition, the ratio of CD4 + / CD8 + cells in the peripheral blood of women RIF +RSA group showed significantly decreased compared to controls.
Conclusion: According to the results, it seems that the increase in T CD8+ cells are more severe in the group with a history of both RIF and RSA.
Keywords: CD8, Recurrent Implantation Failure (RIF), Recurrent Spontaneous Abortion (RSA), T Cell, CD3

P-102: NK Cells Frequency in Peripheral Blood of Women Suffering from Repeated Implantation Failure or Recurrent Miscarriage

Habibi S[1*], Amirchaghmaghi E[1], Mostafaei P[1], Sodeifi N[2], Vesali S[3], Ramezanali F[1]

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. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: habibi.soolmaz@yahoo.com

Background: Reproduction has been one of the most important issues, and recurrent miscarriage (RSA) and repeated implantation failure (RIF), have been main challenges of reproductive medicine and research. However, one of most important interfering factors in successful pregnancy is immune dysregulation. Recent reports show that immune cells undergo significant changes during the menstrual cycle and pregnancy, indicating their role in the reproductive cycle. The aim of this study was to evaluate the frequency of peripheral NK (pNK) cells in women suffering from RIF or RSA.
Materials and Methods: This retrospective study was carried out between July 2018 to January 2020 among women referred to immunology clinic of Royan Institute. Clinical characteristics and findings of immune tests were obtained from 217 women with RIF, 118 women with RSA and 10 women with a history of both RSA and RIF. Also, 10 fertile women with no history of infertility and miscarriage was considered as control group.
Results: The results showed that the frequency of pNK cells (CD16 + CD56 +) phenotypes in women with RIF and RSA were close to each other and slightly lower than the control group. In the RIF + RSA group, decrease of pNK cells was detected in comparison to controls although its decrease was lower than those of the RIF or RSA groups. These differences were not statistically significant.
Conclusion: It seems that the changes in pNK cells are more severe in the group with a history of both RIF and RSA in compare to RIF or RSA ones.
Keywords: CD16, CD56, Natural Killer Cells, Recurrent Implantation Failure (RIF), Recurrent Spontaneous Abortion (RSA)

P-103: Restoration of MEIS1 and KAT2B Genes Expression Levels after Laparoscopic Salpingectomy in Women-with Hydrosalpinx

Hanifeh Z[1, 2*], Zafarani F[3], Ahmadi F[3], Ghaffari F[4], Shahhoseini M[2, 5, 6,]

1. Department of Biology, Faculty of Science, University of Tehran International Kish Campus, Kish, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
6. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: m.shahhoseini@royaninstitute.org

Background: Hydrosalpinx is a disease known with a distally blocked and dilated fallopian tube which has been filled with serous fluid. Evidence suggests that hydrosalpinx is associated with adverse effect on endometrial receptivity by abnormal expression of key molecules such as HOXA10 in the pre-implantation endometrium. Previous data showed salpingectomy results in statistically significant increase in endometrial HOXA10 expression. Complex of HOXA10 with cofactors such as MEIS1 binds to the promoters of their target genes such as KAT2B and promote decidualization of stromal cell. We conducted this study to determine whether salpingectomy could restore the mRNA expressions of MEIS1 and KAT2B in endometrial tissues of patients with hydrosalpinx.
Materials and Methods: Endometrial samples were obtained from 10 patients of reproductive age (20-40 years) with moderate to severe hydrosalpinx proven by hystrosalpingography or laparoscopy who underwent laparoscopic salpingectomy. Mid–luteal-phase endometrial pipelle biopsy performed at the time of surgery and also in forth-post treatment cycle. Also, normal endometrium of ten healthy fertile age-matched women with a history of successful pregnancy as control group collected. All women signed informed consent and had normal hormonal profile, BMI (18–28 kg/m^2) and normal menstrual cycles. RNA extraction and cDNA synthesis were done. Expressions of MEIS1 and KAT2B were determined by quantitative real-time PCR. Data were analyzed based on 2 $-\Delta\Delta$CT to estimate the relative fold change value. Nonparametric Wilcoxon signed rank test was used for data analysis. P value less than 0.05 was considered statistically significant.
Results: There was a significant reduction in the expression levels of MEIS1 and KAT2B gene in the endometrial samples of hydrosalpinx group before surgical removal ($P<0.05$). The obtained data showed that salpingectomy resulted in a 6-fold increase in expression of endometrial MEIS1 gene ($P=0.02$), and a 3.8-fold increase in expression of KAT2B gene ($P=0.039$). Moreover, there was not significant difference in MEIS1 and KAT2B mRNA expression levels between surgical removal group and the controls ($P>0.05$).
Conclusion: Based on our preliminary data it is concluded that laparoscopic salpingectomy could restore the endometrial expression of HOXA10-cofactor- MEIS1 and its target KAT2B in women with hydrosalpinx.
Keywords: Endometrial Receptivity, Hydrosalpinx, Kat2b, Meis1, Salpingectomy

P-104: Nanocarriers Curcumin Pursuit on Efficiency of OVCAR3 Ovarian Cancer Cell Line

Honarmandnia S

Valfajr Health Center, Shiraz university of Medical Sciences, Shiraz, Iran
Email: s.honarmand83@gmail.com

Background: An excessive infirmity is cancer that regularly cured by chemotherapeutic flairs which are poisonous. A basic turmeric (curcumalonga) Curcumin, is equally endowment that is sturdy, economical, and potent responses. Actually, to vanquish these clutches, curcumin was encapsulated in a dendrosomal nanocarier and aftermath this parallel with Oxaliplatin a chemotherapy drug on contemporaries of OVCAR3 ovarian cancer cell line.
Materials and Methods: OVCAR3 cell were cultured in RPMI1640 medium and deal with the dendrosomal curcumin and Oxalipantin at variant admixture for 24, 48 and 72 horus. Current dispute, transpired springed cell viability were tested by MTT method. Also, murrain grade of encapsulated curcumin in nanocarrier of dendrosom was assembled customary curcumin by MTT assay.
Results: The consequences imparted that the dendrosomal cur-

cumin diminished cell commotion in fetal cells as an alliance and time-dependent part equel Oxaliplantin. The IC50 values of dendrosomal curcomin and Oxaliplatin against the OVCAR3 ovarian cancer cell line were resolved as 25,20,15 mg/ml of dendrosomal curcumin and 250,200,150 mg/ml of Oxaliplatin after 24,48,72 hours properly by MTT method. Also balance and morals rate of encapsulated curcumin is excessively greater than natural curcumin.
Conclusion: Dendrosomal nanocarier curcumin could be considered as a probable chemotherapeutic assistant in ovarian cancer such as Oxaliplatin.
Keywords: Dandrosomal Nano Carior Curcumin, Ovarian Cancer, Oxaliplatin

P-105: It's Not Just about The Money: Iranian Gamete Donors' Motivations for Donation

Iranifard E[1*], Ebrahimzadeh Zagami S[2, 3], Amirian M[4], Latifnejad Roudsari R[2, 3]

1. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
2. Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Obstetrics and Gynecology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: rlatifnejad@yahoo.com

Background: Gamete donors often have multifaceted, complex, and diverse motivations for participating in third-party reproduction treatment. Identifying donation motivations is an important issue for the donor recruitment process and also for providing appropriate counseling for donors. The aim of this study was to explore Iranian gamete donors' motivations for donation.
Materials and Methods: A descriptive qualitative study was conducted with six individuals including one embryo donor, two known egg donors, two commercial egg donors, and one commercial sperm donor in two Iranian fertility centers. Participants were recruited through purposive sampling between October 2022 and April 2023. The data were collected using semi-structured interviews. Using MAXQDA 2020 software data analysis was done based on the conventional content analysis approach.
Results: Six subcategories were identified for the main category of "donation motivations". Subcategories included "spiritual beliefs", "benevolence and altruism", "sharing the experience of childbearing with the recipient", "understanding the emotional pain of infertility", "awareness of social pressures due to infertility", and "financial gain".
Conclusion: Donation motivations cannot be identified by a single reason. It is important to notice that every donor has a set of motivations for donating gametes. Infertility treatment centers must take all these motivations into consideration in order to provide better care not only for the donors but also for all those involved in third-party reproduction procedures.
Keywords: Embryo Donation, Motivation, Oocyte Donation, Sperm Donation, Third-Party Reproduction

P-106: Survey Anti-Müllerian Hormones with Metabolic Syndrome in Polycystic Ovary Syndrome

Kalhor M[*], Moini A[2, 3], Jahanian Sadatmahalleh Sh, Qiyasvand S , Nasiri M[2]

1. Department of Reproductive Health and Midwifery, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Gynecology and Obstetrics, Arash Women's Hospital, Tehran, University of Medical Sciences, Tehran, Iran
Email: Shahideh.Jahanian@modares.ac.ir

Background: Polycystic ovary syndrome (PCOS) is a common chronic disease. Metabolic syndrome is one of the most important complications of PCOS. There is a correlation between anti-Müllerian hormones (AMH) and metabolic syndrome. The present study examines this relationship.
Materials and Methods: The present cross-sectional-analytical study was conducted on 61 girls less than 18 years with PCOS in Arash Hospital from 2020 to 2022. Sampling was done by the available methods. Data was collected through a questionnaire including demographic and laboratory information. Statistical analysis was done using SPSS software.
Results: The results showed that the AMH hormone had a statistically significant difference with the metabolic syndrome and non-metabolic syndrome groups ($P<0.001$). So that with the increase of AMH hormone level, the chance of getting metabolic syndrome increases (OR=1.48, P=0.004).
Conclusion: Regarding that the level of AMH hormone had a statistically significant relationship with metabolic syndrome; it can be used in the early diagnosis of PCOS.
Keywords: Anti-Müllerian Hormone, Metabolic Syndrome, Polycystic Ovary Syndrome

P-107: The Assessment of Trans Fats Compounds on Female Infertility, Pregnancy and Abortion

Kheirkhah Rahimabad K[1*], Amiri M[2]

1. Department of Environmental Health, Sibe Salamat Public Health Institute, Tehran, Iran
2. Department of Hotel Management, Allameh Tabatabaei University, Tehran, Iran
Email: kave.kheirkhah@gmail.com

Background: Lifestyle changes over the years and expanded fast foods and a generally unhealthy diet causes damage to the reproductive potential of women, and the rise of infertility among them. A small quantity of Trans fat is found naturally in foods usually in animal products but the vast majority of trans fats are artificial and come from the partially hydrogenated oil found in packaged foods.
Materials and Methods: We followed a cohort study of 544 women a history of infertility 2 years as they tried to become pregnant or became pregnant. Retrospective study comparing dietary data on TFAs and total calories from Block 98 quantitative food frequency questionnaire on these women. We evaluated evidence from TFA and CHD risk controlled feeding trials evaluating risk factors and long-term observational studies evaluating clinical outcomes. A dietary score based on these factors previously related to lower ovulatory disorder infertility and other lifestyle information was prospectively related to the incidence of infertility.
Results: Studies released shows that foods with Trans fats in-

crease 67 percent the risk of ovulatory infertility. Each 2 percent increase in calories from trans fat was correlated with a 73 percent increased risk of ovulatory infertility. 58 percent of babies whose mothers dietary intake of Trans fatty acids 35/2 grams per day, especially in the second and third trimester of pregnancy were born with high birth weight and approximately 26 percent of these mothers were diagnosed with gestational diabetes.
Conclusion: Trans fat consumption, may lead to birth weight. In addition Trans fats cause the negative impact on mother and fetus health. Reports on the harmful action of Trans fats on humans persuasively reveal the need to limit their intake.
Keywords: Abortion, Epidemiology, Infertility, Pregnancy, Trans-Fatty Acids

P-108: Qualitative Exploration of Fears and Concerns of Iranian Gamete Donors: An Interim Analysis

Latifnejad Roudsari R[2, 3*], Iranifard E[1], Ebrahimzadeh Zagami S[2, 3], Amirian M[4]

1. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
3. Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Obstetrics and Gynecology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: rlatifnejad@yahoo.com

Background: Despite the increased demand for donated gametes in third-party reproduction in recent years, the number of donors does not meet the current needs. Among the reasons for this shortage are the obstacles faced by gamete donors. The aim of this study was to explore the obstacles in donation faced by Iranian gamete donors.
Materials and Methods: A descriptive qualitative study was conducted in two fertility centers located in the Northeast and central regions of Iran over a six months period. Using purposive sampling with the maximum variation approach one embryo donor, four oocyte donors, and one sperm donor participated in the study. The data were collected through semi-structured interviews and analyzed by Graneheim and Lundman's conventional content analysis approach using MAXQDA software.
Results: The main category of "fear of donation consequences" emerged. Six subcategories including "fear of clinical procedures", "fear of physical side effects", "fear of social misjudgments", "fear of disclosure outcomes", "psychological, moral and legal concerns for the offspring", and "concerns regarding complying with religious law" were identified.
Conclusion: Identifying and managing fears and concerns of donation for gamete donors is an important step in order to provide quality care for them and as a consequence improve the donation experience of the donors.
Keywords: Embryo Donation, Fear, Oocyte Donation, Sperm Donation, Third-Party Reproduction

P-109: The Impact of Adenomyosis on IVF/ICSI Treatment Outcome in Infertile Women

Madani T[1*], Ahmadi F[2], Jahangiri N[1], Yahyaei A[1], Sanati A[1], Chehrazi M[3]

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. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: tahereh.madani@gmail.com

Background: Adenomyosis is a gynecological disorder suggested to affect 50–85% in women with infertility. In this study, an attempt was made to compare the treatment cycle outcome of assisted reproductive methods in infertile women with adenomyosis and without adenomyosis.
Materials and Methods: This prospective observational study was conducted between 2015 and 2021 on 488 women with adenomyosis (n=53) and without adenomyosis (n=435) who underwent IVF/ICSI treatment for the first time. In this study, patients with adequate ovarian reserve, age < 40 years, body mass index < 30 kg/m^2, Long GnRH agonist protocol or antagonist, and women with embryos with excellent and good grades were included in the study. The patients with a history of previous uterine surgery, uterine fibroid with pressure effect, endometriosis, hydrosalpinx were excluded from the study. The primary outcome measure was live birth and secondary outcome measures included implantation rate, clinical pregnancy and miscarriage rate. SPSS software was used for data analysis. In all tests, a significance level of less than 0.05 was considered.
Results: Both groups were comparable in terms of body mass index, duration of infertility, type of infertility, number of retrieved oocytes, number of MII, fertilization rate, number and quality of retrieved embryos, number and quality of embryos transferred and endometrial thickness on hCG day. The mean age was significantly higher in the adenomyosis group than in the non-adenomyosis group (34.2 ± 4.5 vs. 30.7 ± 4.4 years, P<0.001). Based on the results, there were no significant differences between groups with regards to clinical pregnancy rate, implantation rate, live birth rate and abortion rate.
Conclusion: Adenomyosis appears to be unrelated to clinical pregnancy, implantation, and live birth rates in women underwent IVF/ICSI cycles. A large-scale well-designed prospective study is needed to evaluate the true effect of adenomyosis on fertility outcomes.
Keywords: Adenomyosis, Infertility, Outcome Ultrasound Diagnosis

P-110: The Effects of Autologous Platelet-Rich Plasma on Pregnancy Outcomes in Patients with Unexplained Repeated Implantation Failure Undergoing Fresh or Frozen Embryo Transfer; A Randomized Controlled Trial

Mashayekhy M[1*], Madani T[1], Vesali S[2], Yahyaei A[1]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: azar.yahyaei@yahoo.com

Background: This study aimed to evaluate the effectiveness of intrauterine infusion of platelet-rich plasma (PRP) in improvement of pregnancy outcomes in patients with unexplained RIF undergoing fresh or frozen embryo transfer (ET).

Materials and Methods: In this randomized controlled trial, a total of 80 eligible patients were randomly allocated into the intervention (PRP) and control groups. The intervention group received an intrauterine infusion of 0.8-1 ml PRP at 4–6 times higher concentration than peripheral blood 48 h before ET. The control group underwent standard protocol. All patients were followed up until the study endpoints that included the number of neonates born and pregnancy-related complications.
Results: The clinical pregnancy, live birth, and healthy baby rates were higher in the PRP group than control group (55.8% vs. 20%; 52.9 vs. 8.6%; 47.06 vs. 8.6%; P value<0.000, respectively).
Conclusion: Based on the present study, intrauterine infusion of 0.8-1 ml of PRP, 36-48 hours before blastocyst ET (Fresh or Frozen ET) recommended as an efficient solution in the treatment of patients with unexplained RIF.
Keywords: Autologous Platelet-Rich Plasma, Embryo Transfer, Pregnancy Outcome, Randomized Controlled Trial, Unexplained Repeated Implantation Failure

P-111: The Effect of Hydroalcoholic Extract of Bladder Cherry on Progesterone and Estradiol in NMRI Mice with Polycystic Ovary Syndrome

Mirzapour M*, Zeinali H, Azarnia M

Department of Animal Biology, Kharazmi University, Tehran, Iran
Email: mirzapour.mobinaa@gmail.com

Background: Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders in women of reproductive age. Among the factors involved in PCOS are the abnormal function of estrogen and progesterone hormones. Estradiol (E2) is the most potent form of the hormone estrogen. In this study, changes in progesterone and E2 levels in NMRI mice with PCOS treated with hydroalcoholic extract of bladder cherry (Physalis alkekengi) were investigated.
Materials and Methods: Animals were randomly divided into 6 groups: control; model (PCOS induction by estradiol valerate); extract 1 and 2 (respectively, gavage of 7.5 and 9 grams of bladder cherry extract per kilogram of body weight for 30 days); Experiment 1 and 2 (treatment with extract after induction of PCOS). The serum concentration of hormones was measured by ELISA method. Data were analyzed using one-way ANOVA method.
Results: In the model group, the highest level of E2 (844.70 ± 7.77 pg/ml) and the lowest level of progesterone (1.96 ± 0.10 ng/ml) were observed. Treatment with a dose of 9 g/kg of cherry extract led to a significant decrease in estradiol (156.60 ± 42.52) and a significant increase in progesterone (8.66 ± 0.06) compared to the model.
Conclusion: The results of study indicated the improvement of progesterone and estradiol serum levels in mice with PCOS, and this improvement is probably dose-dependent.
Keywords: Bladder Cherry, Estradiol, Physalis Alkekengi, Polycystic Ovary Syndrome, Progesterone

P-112: COVID-19 and Reproductive Health, Cognizing on Organ Receptors, Polycystic Ovary Syndrome, Insulin Resistance, and Infertility

Mohamadpour M*, Pouraghajan Kh

Department of Science, Vitabiotics Pharmed Company, Tehran, Iran
Email: s.pouraghajan@gmail.com

Background: The reported pathophysiology of COVID-19 in the human reproductive system has led to numerous hypotheses regarding infertility alterations in many aspects. The direct impact of SARS-CoV-2 on the urogenital organs is yet to be explored to implement the correct policies to curb new virus-related ailments. In this study, we analyzed the relationship between known receptors, insulin resistance, and their potential effects on the male and female reproductive system and fertility.
Materials and Methods: We systematically searched for research, cohort, cross-sectional, case–control studies, as well as our own case series for COVID-19-related reproductive system dysfunction. Article screening and data extraction were performed independently by the two authors. Through serum hormonal and biochemical assays of patients, including insulin, fasting blood sugar (FBS), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), in addition to homeostatic model assessment for insulin resistance (HOMA-IR), androgens (testosterone and free androgen index (FAI)), and serum prolactin, we confirmed our research findings.
Results: We found abrupt amounts of serum testosterone, free androgen, prolactin, and biochemical indicators in COVID-19 post-infection side effects related to infertility. By enhancing these factors within the normal range, treatment outcomes are significantly enhanced.
Conclusion: Current investigations targeting COVID-19 and its impact on the reproductive system have revealed a direct relationship between human reproductive problems resulting from SARS-CoV-2 infection and insulin resistance comorbidity with hyperprolactinemia. Regulating testosterone and free androgen index, and especially prolactin, can be significantly helpful for infertility amelioration correlated with insulin resistance.
Keywords: COVID-19, Infertility, Insulin Resistance, PCOS, Reproductive System

P-113: Relationship between Type D Personality and Depression with Endometriosis in Employee Women (Fertile and Infertile)

Navid B[1*], malekzadeh F[2], Moini A[2, 3, 4], Mohammadi M[5]

1. Department of Psychology, Payame Noor University, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Gynecology and Obstetrics, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran
4. Breast Disease Research Center (BDRC), Tehran University of Medical Sciences, Tehran, Iran
5. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Emails: a_moini@royaninstitute.org, f.malekzadeh@royaninstitute.org

Background: Endometriosis is one of the most common gynecological diseases, affecting ~10% of women in reproductive age. Symptoms of endometriosis often affect the psychological and social functioning of patients. According to the multifactorial risk of endometriosis, the aim of this study was to investigate the relationship between depression, type D personality and endometriosis in employee fertile and infertile women.
Materials and Methods: This cross-sectional study was conducted in Tehran, Iran from October 2019 to March 2020. The

study sample consisted of four groups of fertile and infertile women with and without endometriosis. Data was analyzed by Chi-Square, ANOVA, using SPSS Version 22 (Inc. Chicago, IL, USA). The level of statistical significance is 0.05.
Results: In this study, 700 women with an average age of 35.20 ± 5.70 years were evaluated that 99.4% of them have type D personality. The results of our study showed there was no significant difference between depression and endometriosis in four groups of women (fertile and infertile with endometriosis and fertile and infertile without endometriosis). In addition, in 2 subscales of type D personality (negative affect and social inhibition), the highest average score of negative affect was 88.51 ± 7.76 in fertile with endometriosis group and social inhibition was 98.71 ± 5.80 in fertile without endometriosis and this difference was statistically significant (P < 0.001). However, in general, there were no significant differences between type D personality with endometriosis in four groups of women (P= 0.69).
Conclusion: In this study, the majority of women had type D personality which is a negative psychological factor that could be due to the role of women in our society, occupation, marital status, and infertility which are stressful events in women's lives. The management of the mentioned factors is probable to have a lot of psychological effects on women.
Keywords: Depression, Endometriosis, Infertility, Type D personality

P-114: Establishing An Optimal Model of Premature Ovarian Failure in Mice Using Cyclophosphamide

Noori M[1, 2*], Tavana S[1], Cheraghi H[2], Fathi R[1], Mashayekhi M[3]

1. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: s.tavana@royan-rc.ac.ir

Background: Cyclophosphamide (CTX) is a widely used chemotherapeutic drug most likely to affect the ovaries adversely. This study aimed to establish the most effective model of premature ovarian failure (POF) in mice using several different dosages of CTX.
Materials and Methods: The mouse POF model was induced by injecting CTX of 100 mg/kg and 200 mg/kg in one single dose, and 50 mg/kg,100 mg/kg, and 200 mg/kg, in 3 doses (every two days) to NMRI female mice aged between 6-8 weeks, intraperitoneally. Control group was received normal saline instead of CTX. Cycle length, cyclicity pattern (regular/ irregular), ovarian index, follicle counting, and hormone assessment (E2, FSH) were performed 14 days after the last injection of CTX/normal saline.
Results: The cycle length increased from 4.5 to 6.97 at 200 mg/kg (3 doses) in comparison with the control group. Cyclicity pattern in 200 mg/kg (3 doses) was totally irregular in contrast to the control group. Ovarian index and hormonal assessment have no significant change among the groups. Primordial follicle or ovarian reserve in the dose of 200 mg/kg (3 doses) was fewer than the chemo therapied groups but not significant compared to the control group.
Conclusion: The results indicated that the CTX should be administered at a dosage of 200 mg/kg (3 doses) for at least two weeks to establish the most effective POF mouse model.
Keywords: Cyclophosphamide, Mice, Model, Premature Ovarian Failure

P-115: The Effect of Atorvastatin on Histology of Ovaries in Rats Following Induced Primary Ovarian Insufficiency by Cyclophosphamide: A Stereological Study

Notghi P*, Shariatzadeh S M A, Soleimani Mehranjani M

Department of Biology, Faculty of Sciences, Arak University, Arak, Iran
Email: pn_biology@yahoo.com

Background: One of the causes of infertility in women is primary ovarian insufficiency (POI), which causes a decrease in follicular reserve and early menopause in women, before the age of 40. Due to the production of free radicals and oxidative stress, Cyclophosphamide (CTX) causes a decrease in follicular reserve and primary ovarian insufficiency. Due to its pleiotropic effects, Atorvastatin is widely used in the treatment of cardiovascular diseases. In this study, the effect of Atorvastatin on ovarian tissue in rats after induction of primary ovarian insufficiency (POI) by Cyclophosphamide was investigated.
Materials and Methods: Twenty-four female Wistar rats were randomly divided into 4 groups: Control, POI, Atorvastatin, and POI + Atorvastatin (n=6). POI was induced by IP injection of CTX (50 mg/kg/b. w on the 1st day and 8 mg/kg/b.w for 14 consecutive days). Atorvastatin and POI+ Atorvastatin group received 10 mg/kg b.w of Atorvastatin IP injection for 10 consecutive days. Ovaries were removed and fixed for stereological evaluations. The data were analyzed using SPSS software and one-way ANOVA and Tukey tests at a significance level of P<0.05.
Results: The average volume of the total ovary, the volume of the cortex and the volume of the medulla (P<0.01) and the average number of different types of follicles (P<0.001) in the POI group decreased significantly compared to the Control group. While, in the POI+atorvastatin group, there was a significant increase in the total volume of the ovary, the volume of the cortex and the volume of the medulla (P<0.05), the number of primordial and primary follicles (P<0.001), the number of preantral and antral follicles (Pp<0.01) in A comparison with the POI group was observed. There was no significant difference between total ovarian volume, cortex volume, medulla volume (P>0.05), and the average number of primordial and primary follicles in the POI+ Atorvastatin group compared to the Control group (P>0.05).
Conclusion: Atorvastatin can increase follicular reserve and improve the ovarian structure.
Keywords: Atorvastatin, Cyclophosphamide, Primary Ovarian Insufficiency, Rat, Stereology

P-116: Spirituality and Systemic Lupus Erythematosus: A Qualitative Study

Rezaei E

Department of Reproductive Health, Faculty of Nursing and midwifery, Tabriz University of Medical Sciences, Tabriz, Iran
Email: rezai520@yahoo.com

Background: Lupus erythematosus (SLE) is chronic disease that mostly affects women of the reproductive age. On the other hand, spiritual health empowers women to tolerate chronic disease easily. This study was conducted with the aim of exploring the perceptions and the viewpoints of women with SLE regarding spirituality.
Materials and Methods: This qualitative research was conducted using 27 semi-structured deep interviews with 19 married women suffering from SLE (15-49 years old) selected through purposive sampling in the referral Rheumatology Center in Iran. Data analysis was performed with a content analysis approach using the conventional method proposed by the Zhang and Wildemuth (2016) by 10 MAXQDA
Results: The women's perceptions about spirituality were categorized in two subcategories included: 1. forgiveness of sins (According to the participants, repenting for sins is a way of mitigating sins and approaching God) and 2. resorting to God (that is the key to locked doors and a facilitator for the hopeless when they are deep in crises. Through thanksgiving and with the help of God, they can prevent falling into the abyss of sins under the effect of spiritual beliefs. Many participants considered the disease as a way to approach God, and spiritual beliefs were regarded as a mediator to this end).
Conclusion: This study emphasized to spiritual believes as an important way to tolerate and cope with difficulties of SLE in women. It is hoped that through this research, one can take a step to enhance the awareness of care providers about spiritual factors.
Keyword: Qualitative Study, Systemic Lupus Erythematosus, Women

P-117: Evaluation of The Anti-Implantation and Abortifacient Effects of Pea Extract on Pregnant Rats

Shaaban Z[1*], Tansaz M[2], Jafari F[3]

1. Department of Animal Sciences, College of Agriculture, Shiraz University, Shiraz, Iran
2. Department of Traditional Medicine, School of Traditional Medicine, Sahid Beheshti University of Medical Sciences, Tehran, Iran
3. Traditional Medicine & Materia Medica Research Center, Tehran, Iran
Email: zshaaban412@gmail.com

Background: Spontaneous abortion is one of the most common complications of pregnancy for couples expecting a child. Chickpeas or hummus have an abortifacient effect from the point of view of Iranian Traditional Medicine. Therefore, the study aimed to investigate the abortifacient effect of chickpea extract on pregnant rats.
Materials and Methods: Adult female rats were mated with sexually experienced males overnight and the next day were evaluated to determine gestation day 1(GD1). Pregnant Wistar rats (n=66) received pea extract orally at doses of 500 and 1000 mg/kg body weight (BW) one week before mating (1 WBM), from the mating day (MD), from the first day of pregnancy (GD1), during pregnancy (3 GD/W), and days 13 to 15 of pregnancy (GD 13-15) (n=6 per group) until the time of dissection (end of pregnancy (day 21)) except for groups 3 GD/W and GD 13-15, which were dissected on days 18 and 16 of pregnancy, respectively. The sham group (n=6) received vehicle only (normal saline).
Results: The number of live fetuses at 1000 mg/kg chickpea extract was lower in 3 GD/W, MD, and GD 13-15 groups, respectively, compared to the control group (P=0.0007). The number of non-viable fetuses was higher in the 1000 mg/kg GD 13-15 group compared to the control group (P=0.0005). The serum progesterone concentration was lower in the 500 mg/kg in MD and GD1 groups than in the control group (P=0.0063). Administering a dose of 500 mg/kg BW before pregnancy and during pregnancy didn't affect the number of viable and non-viable fetuses.
Conclusion: A high dose of chickpea extract in the critical days of mid-pregnancy, before pregnancy, and three times a week during pregnancy has adverse effects on the survival of embryos in rats. Also, long-term administration from one week before mating and throughout pregnancy can reduce serum progesterone concentration.
Keywords: Abortion, Hummus, Iranian Traditional Medicine, Miscarriage, Persian Medicine

P-118: Comparison of The Effect of Cervical Dilatation in The Two Stages of The Onset of The Long OCP Cycle and The Onset of Stimulation on Pregnancy Outcomes in Individuals with Difficult Embryo Transfer in IVF/ ICSI Cycles, at The Royan Institute

Shamloo Ahmadi F[1, 2*], Arabipoor A[2], Ghaffari F[2]

1. Department of Developmental Biology, Faculty of Basic Science Technology, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: ghafaryf@yahoo.com

Background: The most debated subject matter in assisted reproductive technology (ART) is how to improve implantation and pregnancy rates. Difficult embryo transfers were associated with lower live birth rates but no difference in ectopic pregnancy or miscarriage rates while the presence of blood can indicate a relatively more traumatic and difficult transfer, it does not necessarily lead to a lower birth rate. The most challenging debate is what is the optimum time for cervix dilatation before ET. The time of doing it is not well known. The purpose of this study is to investigate the effect of performing cervical dilatation at two different time points in patients who had a history of difficult embryo transfer at the Royan Institute, and returned for a further ART cycle.
Materials and Methods: In this retrospective study all patients who had difficult ET in the Royan Research Institute from 2009 to 2014 and had at least one grade A or grade B embryo participated in the study and were divided into two groups. The eligible patients entered the study allocated into 2 groups (dilation before the start of stimulation or dilation before the OCP long cycle). For all patients, cervical dilatation was done by the use of Bougei tube number 8 under general anesthesia. The embryo transfer procedure was performed by a specialist doctor for all patients. Following dilatation, the degree of difficulty of ET and pregnancy rate were compared between groups.
Results: Finally, 26 patients in group 1 (cervical dilatation at the beginning of OCP Long) and 28 patients in group 2 (cervical dilatation at the beginning of ovarian stimulation) had the embryo transfer and the results of their cycle were analyzed and compared. Comparison of initial and clinical characteristics of patients between groups showed that patients in the two groups were homogeneous. Analysis of the results of ovarian stimu-

lation and ET cycle demonstrated that the duration of ovarian stimulation, number of used of gonadotropin ampules c, endometrial thickness on trigger day, number of obtained, MII oocytes and embryos transferred, type of embryo transfer was not statistically significant. Comparison of the degree of difficulty of ET between groups showed that the number of cases of easy transfer in group 1 (cervical dilatation at the beginning of OCP Long) is significantly higher than group 2 (cervical dilatation at the beginning of ovarian stimulation) (80.8 vs. 60.6%) (P = 0.04). In the following, the clinical pregnancy and live birth rates were higher in group 1 than group 2; however, the difference between groups was not statistically significant (P= 0.1 and P= 0.2, respectively). Also, the rates of chemical pregnancy and abortion was not significantly different between groups.
Conclusion: According to the results of the present study, the longer distance between dilatation and ET (beginning of the long OCP cycle), was associated with greater rate of easy ET and unassisted embryo transfer (needle, tenaculum and hysterometer....). The pregnancy results were more favorable, however more studies are needed in this area.
Keywords: Cervical Dilatation, Difficult Embryo Transfere, Pregnancy Rate

P-119: Altered Expression of Glycodelin A, Heparin Binding Epidermal Growth Factor and InterleukinL-6 in Endometrium of Women with Hydrosalpinx: A Case-Control Study

Shamlou N[1*], Kazerouni F[1], Ghaffari F[2], Aflatoonian R[2], Chekini Z[2], Aghajanpour S[2], Faridi M[3]

1. Department of Laboratory Medicine School of Allied Medical Sciences, Shahid Beheshti 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 Surgery, Iranmehr Hospital, Tehran, Iran

Email: f_kazerouni@sbmu.ac.ir

Background: Hydrosalpinx, as one of the known diseases of the fallopian tubes, reduces the implantation rate and success in Invitro Fertilization (IVF) and exhibit its destructive effects by affecting the endometrial receptivity. In this study, we amid evaluate the mRNA expression of Interleukin-6(IL-6), Heparin Binding –Epidermal Growth Factor(HB-EGF) and Glycodelin A(Gd A) genes in endometrium of women with hydrosalpinx.
Materials and Methods: This case-control study was performed from2018 -2020 years in Royan Institute. Ten patients with hydrosalpinx before salpingectomy and ten fertile women on days 19 to 24 of the menstrual cycle volunteered for uterine endometrial sampling and mRNA expression of IL-6, HB-EGF and GdA genes were analyzed by Quantitative Polymerase Chain Reaction (QPCR).
Results: mRNA expression of IL-6 showed a significant increase in patients with hydrosalpinx compared to the fertile group, but the decrease in HB-EGF and GdA gene expression, although expected, did not show a significant difference between the two groups.
Conclusion: Although just IL-6 expression was significantly elevated, and HB-EGF and GdA reduction was not statistically significant between the case and the control groups, but it seems that all three of these genes are important in endometrial receptivity and the changes in their expression could be due to the presence of hydrosalpinx.

Keywords: Glycodelin A, HB-EGF, Hydrosalpinx, Interleukin-6 Expression

P-120: Repro-AI: Current Status and Future Perspectives of AI in ART

Vaseghi Dodaran H[1*], Atshan M[2], Moharramnia H[3]

1. CEO, RADIN PAZHOH PARS AZMA Company, Ardabil Science and Technology Park, Ardabil, Iran

2. Department of Biology, Science Faculty, Arak University, Arak, Iran

3. Department of Computer Engineering, Technical and Engineering Faculty, Payam Noor University, Ardabil, Iran

Email: h.vaseghi28@yahoo.com

Background: Infertility rate in the world varies from 10 to 22%. However, couples receive successful infertility treatment at low rates, leading to repeat treatment or treatment withdrawal. Since the birth of the first IVF baby in 1978, more than eight million babies have been born as a result of the assisted reproductive technique. Artificial intelligence is rapidly changing the practice of medicine in various fields. Artificial intelligence entered the research world of assisted reproductive technologies (ART) in the late 1990s with the creation of an algorithm aimed at predicting the outcome of IVF. In reproductive medicine, artificial intelligence can significantly reduce the highly manual and labor-intensive processes of ART. The aim of this paper is to provide a systematic review to establish the actual contribution of artificial intelligence for predicting ART outcomes.
Materials and Methods: The PubMed database was searched for citations indexed with “artificial intelligence” and at least one of the medical subject heading terms between January 1, 2000 and April 30, 2020: “artificial intelligence”. "Obstetrics and Gynecology"; "Assisted Reproductive Techniques, "or "Fertility"
Results: The PubMed search retrieved 750 citations and 55 publications met the selection criteria. All ART subdomains were covered. Among these 55 articles, 15 were related to embryo selection, 25 were sperm evaluation, and 15 were related to egg selection and implantation technologies. We observed a generally increasing trend in AI-related publications in assisted reproductive techniques over the past two decades.
Conclusion: The development of new artificial intelligence frameworks to predict the ideal outcome in reproductive medicine is a necessity. As a comprehensive result, this new system can reduce the instability between observers, reduce risks during egg stimulation, reduce close and personal clinical contacts, and from the financial aspect, increase clinical profitability and better determination of sperm tests and evaluation of egg quality and embryo selection.
Keywords: Assisted Reproductive Technology, Artificial Intelligence, Fertility, Machine Learning

P-121: Laparoscopic Salpingectomy Restores Loss of HOXD10 Expression Induced by Upregulation of Long Non-Coding HOX Transcript Antisense Intergenic RNA (HOTAIR) in Women with Hydrosalpinx

Zafarani F[1, 2*], Soleimani Mehranjani M[1], Ghaffari F[3], Shahhoseini M[4], Shariatzadeh SMA[1], Ahmadi F[2], Lotfi M[5]

1. Development of Biology, Department of Biology, Faculty of Science, Arak University, Arak, Iran

2. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Reproductive Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
5. Department of Pathology, Amir Aalam Hospital, Tehran University of Medical Sciences,Tehran, Iran
Email: fzafarani1391@gmail.com

Background: The deleterious influence of hydrosalpinx on endometrial receptivity biomarkers such as Homeobox gene A10, Leukemia inhibitory factor and integrinshas been demonstrated. Moreover, the presence of local inflammatory mediators such as IL-2, IL-6 and TNF-αin the endometrium exposed to hydrosalpingial fluid have been established. We investigated the expression levels of HOXD10 and HOTAIR mRNA in endometrium of infertile women with hydrosalpinges compared to controls. We next determined the correlation of the expression level of these genes before and after salpingectomy.
Materials and Methods: Ten infertile women with moderate to severe hydrosalpinxes who underwent laparoscopic salpingectomy, were recruited. Mid–luteal-phase endometrial sampling was performed at the time of surgery and patients were followed up for second endometrial biopsies in the mid-luteal phase of forth-post treatment cycle. Ten healthy fertile women with successful pregnancy were considered as control and underwent endometrial biopsy in mid-luteal phase. Control and hydrosalpinx endometrial samples were obtained by pipelle. After RNA extraction and cDNA synthesis, real-time PCR was used for quantitative HOXD10 and HOTAIR gene expression levels. Wilcoxon signed Ranks Test and kruskal-Wallis were used for data analysis. P-value less than 0.05 was statistically significant.
Results: Salpingectomy significantly increases HOXD10 expression level (P=0.006) and decreases HOTAIR expression levels (P= 0.01). Moreover, there was no correlation between HOXD10 and HOTAIR expression levels (r= - 0.18, P=0.3) before salpingectomy.
Conclusion: Hydrosalpinx removal restores HOXD10 and down-regulates HOTAIR expression levels. This may be a mechanism by which salpingectomy improves implantation rate in these patients.
Keywords: Endometrial Receptivity, HOXD10 Expression, HOTAIR, Hydrosalpinx, Salpingectomy

P-122: The Possible Association between An Empirical Dietary Inflammatory Pattern (EDIP) and Risk of Polycystic Ovary Syndrome: A Case Control Study.

Zirak Sharkesh E[1*], Keshavarz A[2], Nazari L[3], Abbasi B[1]

1. Department of Nutrition, Electronic Health and Statistics Surveillance Research Centre, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Department of Nutrition and Biochemistry, Faculty of Public Health, Tehran University of Medical Science, Tehran, Iran
3. Department of Obstetrics and Gynecology, Preventative Gynecology Research Center , Shahid Beheshti University of Medical Science, Tehran, Iran
Email: abbasi.b@srbiau.ac.ir

Background: Polycystic ovary syndrome (PCOS) as the most common endocrine disorder in women of reproductive age is influenced by various factors. Since there are some evidences linking inflammation with chronic diseases, the aim of this study was to investigate the possible association between an empirical dietary inflammatory pattern score (EDIP) and risk of PCOS in a case control design study.
Materials and Methods: This case–control study was conducted on Tehranian women population, Iran. A total of 496 participants (203 women with PCOS in case group and 291 healthy people for the control group), ages 18 to 45 years were recruited in the study. Demographic information, anthropometric indices, physical activity level, and dietary intake were collected by a trained nutritionist. EDIP score was calculated to estimate overall dietary inflammatory potential based on 18 food groups intake. Statistical analysis was performed with SPSS version 19.
Results: Based on the results, the mean age of participants in case and control groups were 28.98 ± 5.43 and 30.15 ± 6.21 years, respectively. Individuals with PCOS had significant higher difference in EDIP score compare to healthy participants (2.03 ± 1.13 vs 1.70 ± 0.93, P< 0.001). Also, the odds ratio (OR) and 95% confidence interval (CIs) for the risk of PCOS across quartiles of EDIP showed a significant direct relationship (P= 0.003)
Conclusion: In conclusion, our study showed that there was a direct association between PCOS risk and EDIP score. Findings suggest that inflammatory index might be a potential mechanism linking diet and PCOS development.
Keywords: Inflammation, Inflammatory Markers, Polycystic Ovary Syndrome, Women

Genetics

P-123: Evaluation of Genetic Variations of Exon 4 of The AURKC Gene in Patients with Macrocephalic Spermatozoa Referring to Royan Institute

Loghmani F[1*], Siadat SF[1], Hosseini SH[2], , Sabbaghian M[2]

1. Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: Macrocephalic sperm have an abnormally large head and/or multiple flagella. This syndrome is considered to be an autosomal recessive type of teratozoospermia that leads to male infertility. To date, the only gene involves in macrocephalic is the AURKC (Aurora Kinase C gene). Mutation in the AURKC gene causes defects in meiosis, but spermatogenesis is not affected and leads to the production of large-headed sperm.
Materials and Methods: A total of 10 infertile men with macrocephalic sperm syndrome were considered as the case group and 10 men with normal spermogram as the control group. DNA was extracted from the peripheral blood samples of selected individuals. After designing primers, PCR reactions were done and DNA sequencing was performed for PCR products. The results were analyzed by Finch TV and Nucleotide Blast.
Results: Results of sanger-sequencing revealed no mutations in men with macrocephalic spermatozoa syndrome or the control

group.
Conclusion: According to our study, it can be concluded that there is no relationship between the occurrence of sperm macrocephalic syndrome disorder and nucleotide changes in exon 4 of the AURKC gene and it is suggested review other exons and Regulatory regions.
Keywords: AURKC Gene, Macrocephalic Sperm Syndrome, Male Infertility

P-124: Rutin and Cytarabine Effects on GDF9 Gene Expression on Mice Ovary Tissue

Amirnahadehkey N*, Mohseni Kouchesfehani H, Jalali H

Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Email: kouchesfehani@yahoo.com

Background: About one third of the induced infertility was related to effective drugs in leukemia, which can be referred to cytarabine. The oocyte-secreted factors growth differentiation factor 9 (GDF9)plays essential roles in follicle development and oocyte maturation. Decreased expression of genes related to oocyte maturation (Gdf9), which may be caused by impaired steroid hormone synthesis and lipid metabolism, thus inhibiting follicular growth and ovulation. In this research,the effect of Rutin and cytarabine on the ovarian tissue and the expression of GDF9 gene was investigated.
Materials and Methods: 16 adult female mice of BALB/C were divided in four groups:group1:control group,Group2 that received as IP (intraperitioneal injection) 150mg/kg of Rutin that solved in solvent (DMSO+Tween80+saline) every other day for two weeks. Group3 that received 100mg/kg of Cytarabine in the form of IP in single dose.Group4 that received 100mg/kg of Cytarabine in the form of IP in single dose and 150mg/kg of Rutin that solved in solvent (DMSO+Tween80+saline)every other day for two weeks.Then ovarian tissue sections were stained with Hematoxylin-Eosin.Quantitave evaluation of the Gdf9 gene expression has been done with the Real-time PCR method and the data were analyzed with spss software.
Results: All types of follicles decreased in the cytarabine group and increased in the rutin group. Cytarabine and rutin did not affect GDF9 expression($P<0.05$).
Conclusion: Considering that the GDF9 expression did not change,but the number of follicles decreased in the cytarabine group and increased in the rutin group,it is expected that other factors played a role in these ovarian changes, and further investigations are suggested.
Keywords: Cytarabine, GDF9, Rutin

P-125: Evaluation of Genetics Variations of Exon 1 of The AURKC Gene in Patients with Macrocephalic Spermatozoa Referring to Royan Institute

Esmi Y[1*], Siadat SF[1], Hosseini SH[2], , Sabbaghian M[2]

1. Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: marjan.sabbaghian@gmail.com

Background: Macrocephalic is defined as large-headed, multiflagellated spermatozoa in infertile men and results in the father's existence unable to have a biological child. Sperm macrocephalic syndrome is caused by the mutation of the Aurora Kinase C (AURKC) gene situated on the long arm of chromosome 19 at 19q13.43. The AURKC gene encodes the third member of the Aurora subfamily of serine/threonine protein kinases and is usually expressed in the testis. The AURKC gene plays exigent roles in centrosome function, homologous chromosome segregation, and cytokinesis for meiosis.
Materials and Methods: A total of 10 infertile men with Sperm macrocephalic syndrome were considered as the case group and 10 men with normal spermogram as the control group. DNA was extracted from the peripheral blood samples of selected individuals. After designing primers, PCR reactions were done and DNA sequencing was performed for PCR products. The results were analyzed by Finch TV and Nucleotide Blast.
Results: Results of sanger-sequencing revealed no mutations in men with Sperm macrocephalic syndrome or the control group.
Conclusion: According to our study, It can be concluded that there is no relationship between the occurrence of sperm macrocephalic syndrome disorder and nucleotide changes in exon 1 of the AURKC gene and it is suggested review other exons and Regulatory regions.
Keywords: Infertile Men, Sperm macrocephalic syndrome, AURKC Gene

P-126: Pathogenicity Prediction of A Missense Mutation and its Association with Non-Obstructive Azoospermia Based on In-Silico Analysis

Ghorbanian R*, Khatami M

Department of Biology, Yazd University, Yazd, Iran
Email: m.khatami@yazd.ac.ir

Background: Male infertility is a widespread problem affecting nearly 50 million couples worldwide, with about half of the cases originating from male-related issues. Azoospermia, which accounts for 10-15% of male infertility cases, can be caused by genetic mutations in genes related to male fertility, including NANOS2. Recent studies have shown that a missense mutation, His68Gln, in the NANOS2 gene may contribute to the development of azoospermia, particularly Non-Obstructive Azoospermia(NOA), which is characterized by the total absence of sperm in ejaculate without any obstruction in the ejaculatory ducts. In this in-silico study, the effects of this mutations on the molecular mechanisms underlying azoospermia is investigated in order to investigating a molecular biomarker for further research.
Materials and Methods: By utilizing bioinformatic tools such as HOPE, I-Mutant, and PANTHER, the stability, function, and structure of mutant protein is evaluated.
Results: The results showed that the mutation led to a increase in protein stability and changes in protein interactions and functions. Moreover, it is found that this mutation could be considered as potentially damaging, with a probability of deleterious effects, associated with NOA. This mutation was predicted to be disease-related and recommended as a molecular marker for NOA.
Conclusion: Collectively, the findings provide valuable insights into the putative role of the mutation in the pathogenesis of male infertility and may lead to new diagnostic and therapeutic options for affected individuals.
Keywords: Genetic Mutation, Male Fertility, Molecular Bio-

marker, Bioinformatic Tools

P-127: TBL3, MAGEA8 and NTN1 as Hub Scaffold Gene Involved in Sperm of Non-Obstructive Azoospermia

Hashemi Karoii D[1*], Azizi H[2]

1. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Background: During mammalian spermatogenesis, the cytoskeleton system plays a significant role in morphological changes. The formation of abnormally shaped spermatozoa might be caused by a problem with the cytoskeleton system. Male infertility such as non-obstructive azoospermia (NOA) might be explained by studies of the cytoskeletal system during spermatogenesis.
Materials and Methods: The scaffold genes were analyzed by microarray and bioinformatics (300 sperm genes) and we used real time polymerase chain reaction for confirm these genes.
Results: In the microarray analyses of three human cases with different NOA sperm, the expression of TBL3 (transducin beta like 3), MAGEA8 (MAGE family member A8) was upregulated, while expression of NTN1 (netrin 1) was downregulated. A combined analysis of Enrich Shiny Gene Ontology (GO), STRING, and Cytoscape was used to predict proteins' molecular interactions and then to recognize master pathways. Functional enrichment analysis showed that the biological process (BP) mitotic cytokinesis, cytoskeleton-dependent cytokinesis, positive regulation of stem cell proliferation, negative regulation of Rho protein signal transduction, supramolecular fiber organization, negative regulation of Ras protein signal transduction, positive regulation of protein localization to the cell periphery and positive regulation of cell-substrate adhesion were significantly expressed in up/down regulated differentially expressed genes (DEGs) in sperm. In MF experiments of DEGs that were up/down regulated, it was found that GTPase and small GTPase bindings, tubulin bindings, gap junction channels, glutathione transmembrane transport, gap junction hemichannel activity, and tripeptide transmembrane transport were overexpressed.
Conclusion: According to our findings, non-obstructive azoospermia and infertility can be explained by genes and their interacting hub proteins.
Keywords: Gene Expression, Non-obstructive Azoospermia, Microarray, Scaffold

P-128: Comprehensive Bioinformatics Analysis to Reveal Key miRNA and lncRNA Involved in The Sperm of Couples of Recurrent Spontaneous Abortion Based on Target of Paternal Imprinting Genes

Hashemi Karoii D[*], Shahhoseini M[1]

Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: d.hashemi.karoii@ut.ac.ir

Background: Genomic imprinting is defined as an epigenetic modification that leads to parent-of-origin specific monoallelic expression. Some current research on the fetal control growth has been focused on the study of genes that display imprinted expression in utero. Six paternal imprinted genes, MEST/PEG1, PEG9/Dlk1, PEG11/Rtl1, PEG3, PEG5 and PEG6, are well known to play a role in fetal growth and placental development. Recurrent spontaneous abortion (RSA) in the general reproductive population is a very common occurrence and other genetic causes beyond chromosomal abnormalities could be involved in spontaneous miscarriages or fetal deaths, such as alteration of expression in imprinted genes particularly those related to fetal or placental growth.
Materials and Methods: In this study, we use microRNA and lncRNA and PPI database to predict and find hub imprinting gene target. The differential expression of miRNAs and lncRNAs was identified using the DESeq2 package. Functional enrichment analyses and protein–protein interaction (PPI) were executed. Then, the hub genes were filtered and molecular docking analysis was performed.
Results: We revealed that 152 intersected differentially expressed miRNA and 136 intersected differentially expressed lncRNA were shared between the four datasets. we uncovered the top 4 hub miRNA in which mmu-miR-3073-3p, mmu-miR-3061-5p, hsa-miR-598 and mmu-miR-409-5p were significantly affect these target gene. Particularly, lncRNA-mRNA was predicted by molecular docking to be a candidate drug for treating RSA. we uncovered the top 6 hub lncRNA in which LINC01471, FREM2-AS1, LINC02260, LINC00502 and USP27X-AS1 were significantly affect these target gene. Finally, we proposed a potential regulatory mechanism for RSA. Overall, we uncovered a hub miRNA-lncRNA-mRNA network that might underlie a critical posttranslational regulatory mechanism in RSA, in which mmu-miR-3073-3p, mmu-miR-3061-5p, hsa-miR-598, mmu-miR-409-5p, LINC01471, FREM2-AS1, LINC02260, LINC00502 and USP27X-AS1.
Conclusion: These miRNA-lncRNA-mRNA targeted could be valuable biomarkers and provided core RNAs therapeutic targets for RSA and RIF.
Keywords: Biomarkers, lncRNA, miRNA, Recurrent Spontaneous Abortion

P-129: Comparison of Ferroptosis and Autophagy Genes in Menstrual Blood-Derived Mesenchymal Stem Cells between Normal Women and Women with Endometriosis

Hedayatnia H[1*], Kalhor N[2]

1. Department of Biology, Islamic Azad University, Science and Research, Tehran, Iran
2. Department of Mesenchymal Stem Cells, Academic Center for Education, Culture, and Research (ACECR), Qom, Iran
Email: hassanhedayatnia_3@yahoo.com

Background: The etiology of endometriosis is not yet fully understood. Studying how endometriosis develops can help to create better treatments and increase knowledge about the condition and will greatly facilitate its diagnose.
Materials and Methods: Menstrual blood-derived mesenchymal stem cells (MenSCs) of normal women and women with grade III and IV endometriosis were obtained on the second or third day of menstruation. The mononuclear layer of cells was isolated and cultured in DMEM medium containing 10% FBS and 1% penicillin-streptomycin and incubated at 37 ° C and 97% humidity and 5% CO_2.Cell surface markers were assessed by Flow cytometry and , expression of the genes were assessed by real-time PCR.
Results: The investigation found that women with endometriosis had upregulated mRNA expression of Beclin-1 (an

autophagy-related gene) compared to the normal group (P ≤ 0:024). No significant difference was found in the expression of other autophagy-related genes (ATG7, ATG14). TXNRD2 (a ferroptosis-related gene often overexpressed in various cancer types) also showed upregulation (P ≤ 0:000) in women with endometriosis, but no significant difference was detected in the expression of other ferroptosis-related genes (Nrf2, GPX4, TXN2).
Conclusion: We found variations in gene expression levels of ferroptosis and autophagy genes in MenSCs taken from women with endometriosis compared to normal women, suggesting a role in endometriosis development. This supports the retrograde menstruation theory and contributes to Multiomics profiling studies. MenSCs can aid discovery of new drug targets, biomarkers, and non-invasive diagnostic strategies, and predict endometriosis risk in healthy women.
Keywords: Autophagy, Endometriosis, Ferroptosis, Menestrual Blood, Mesenchymal Stem Cells (MSCs)

P-130: The Relationship Between mir-let-7b Expression and Sperm Parameters, Sperm DNA Fragmentation in Oligozoospermic Infertile Patients

Jannatifar R[1*], Shavandi L[2], Jamallo F[2], Sahraei S[1], Piroozmanesh H[3]

1. Department of Reproductive Biology, Academic Center for Education Culture and Research (ACECR), QOM, Iran
2. Biology Department, Islamic Azad University, Qom Branch, Qom, Iran
3. Department of Biology, Arak University, Arak Branch, Arak, Iran
Email: rahiljanati2016@gmail.com

Background: The aim this study the evaulation relationship between mir-let-7b expression and sperm parameters, sperm DNA fragmentation in oligozoospermic infertile patients.
Materials and Methods: In this study, the amount of expression of mir-let-7b was evaluated in oligozoospermic patients group compared to control group. In this study after the separating sperms, total RNA was isolated and then cDNA was synthesized. The expression level of mir- let-7b was evaluated by real time PCR. The DNA fragmentation index was measured using SCD Assay technique. Using WHO (2010) criteria, sperm parameters were evaluated.
Results: Mir-let-7b level was significantly higher in oligozoospermic infertile patients than control group (P=0.009)., Correlation analysis highlighted that sperm count, motility, and morphology were negatively correlated with Mir-let-7b level, but positively correlated with the sperm DNA fragmentation (P<0.05).
Conclusion: The results of this study indicate that miRNA can have a key role in spermatogenesis and might have a diagnostic and prognostic value in men infertility. Changes in mir- let-7b level in oligozoospermic patients may be associated with the susceptibility and progression of infertility.
Keywords: DNA Fragmentation, mir -let-7b, Oligozoospermic, Sperm

P-131: Examining The Relationship between Expression of BAX and BCL-2 Genes and Sperm Parameters , DNA Fragmentation in Oligoasthenoteratozoospermia Men

Jannatifar R[1*], Babaei F[2], Khoshsokhan muzaffar M[2], Sahraei S[1], Piroozmanesh H[3]

1. Department of Reproductive Biology, Academic Center for Education Culture and Research (ACECR), Academic Center for Education Culture and Research (ACECR), QOM, Iran
2. Biology Department, Academic Center for Education Culture and Research (ACECR), QOM, Iran
3. Department of Reproductive Biology, Academic Center for
3. Biology Departmkent, Arak University, Arak Branch, Arak, Iran
Email: rahiljanati2016@gmail.com

Background: The amount of expression of BAX and BCL-2 genes in sperm as well as its association with sperm parameters and DNA fragmentation index in infertile men with oligoasthenoteratozoospermia.
Materials and Methods: A total of 50 men were investigated and divided into the following groups: healthy fertile men (n = 25), and infertile oligoasthenoteratozoospermia men (n = 25). They were subjected to history taking, clinical examination, and semen analysis. Sperm is divided into two parts based on which Real Time-PCR and SCD Assay techniques were conducted. After extracting RNA and producing cDNA, the amount of expression of BAX and BCL-2 genes was measured using Real Time-PCR. The amount of sperm DNA fragmentation was measured using SCD Assay technique. Using WHO(2010) criteria, sperm parameters were evaluated.
Results: The amount of expression of BAX gene was significantly increased, and BCL2 gene was significantly decreased in oligoasthenoteratozoospermia men (P<0.05). The expression of BAX gene demonstrated significant positive correlation with sperm concentration, sperm motility, and sperm normal morphology (P<0.05). The expression of BCL2 gene significant negative correlation with sperm concentration, sperm motility, and sperm normal morphology P<0.05). Also, this study showed that the amount of expression of BAX and BCL-2 were significant correlation with the DNA fragmentation in oligoasthenoteratozoospermia group (P<0.05).
Conclusion: In this study demostrated that amount of expression of BAX and BCL2 gene significantly correlation with sperm parameters, and DNA fragmentation.
Keywords: BAX, BCL-2, DNA Fragmentation, Sperm Parameters

P-132: The Correlation of Sperm MicroRNA-449 and 34c Expression and Sperm Quality in Men with Oligoasthenoteratozoospermia

Jannatifar R[1*], Dujaili F[1], Khoshsokhan Muzaffar M[2], Sahraei S[2], Piroozmanesh H[2]

1. Department of Reproductive Biology, Academic Center for Education Culture and Research (ACECR), Qom, Iran
2. Biology Department, Islamic Azad University, Qom Branch, Qom, Iran
3. Biology Department, Arak University, Arak Branch, Arak, Iran
Email: rahiljanati2016@gmail.com

Background: The aim this study the evaulation relationship between expression of microRNA-449 and 34c in sperm and sperm parameters in oligoasthenoteratozoospermia men.
Materials and Methods: In this study, men referred to infertility treatment center were divided into 2 groups. The control group (n=20) and oligoasthenoteratozoospermia group (n=20). Examination of sperm parameters was detemenated according to WHO (2010) instructions. MicroRNA (miRNA) investigation was performed using in silico predictive analysis, validated

reverse transcription-quantitative PCR (RT-qPCR).
Results: The expression level of Mir-449 was significantly up – regulated, and Mir-34c was significantly down-regulated in oligoasthenoteratozoospermia men compared with age-matched normozoospermic men as determined by RT-qPCR ($P<0.05$). Correlation analysis highlighted that sperm count, motility, and morphology were negatively correlated with miRNA-449and positively correlated with the lower expression level of Mir-34c($P<0.05$). Furthermore, an inverse correlation between higher expression level of miRNA-449and lower expression level of Mir-34c was observed($P<0.05$).
Conclusion: Findings suggest that the higher expression of miRNA-449, and the lower expression of Mir-34c are associated with oligoasthenoteratozoospermia and male infertility, probably through influencing basic semen parameters. This study lay the groundwork for future studies focused on investigating therapies for male infertility.
Keywords: MiR-34c, MiRNA-449, Oligoasthenoteratozoospermia, Sperm Parameters

P-133: Differences in Gene Expression of Enzymes Involved in Insulin Metabolism in Abdominal Subcutaneous Adipose Tissue between Pregnant Women with and without Polycystic Ovary Syndrome

Joodi Alvandi SH[1*], Ghoraeian P[1], Alizadeh A[2], Aghajanpour S[3], Emami N[3], Moini A[3], Shahhoseini M[4*], Hafezi M[3]

1. Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, 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
4. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Background: Women suffering from PCOS (Polycystic ovary syndrome) often exacerbate insulin resistance, which could be due to AT (Adipose tissue) disfunction.A defect in the active PIK3 (Phosphatidylinositol 3-kinase) that is related to insulin receptor signaling was detected in PCOS patients and the catalytic and regulatory subunits of PIK3 gene are PIK3CA (Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) and PIK3R1 (phosphoinositide-3-kinase regulatory subunit 1), Our objective was comparing mRNA levels of two genes PIK3CA and PIK3R1 in subcutaneous AT of pregnant women with PCOS and without PCOS.
Materials and Method: In a case-control study and after a cesarean section, subcutaneous AT were obtained from two groups of women with PCOS (n=12) and without PCOS (non-PCOS; n=24) (1:2 ratio). Then, mRNA extraction and cDNA synthesis were done and the relative expression of genes were evaluated using Real-time PCR.
Results: No significant differences were detected with respect to age, BMI (prior pregnancy and at delivery day) among 2 groups. The mRNA expression levels of PIK3R1($p<0.05$) and PIK3CA ($p<0.05$) were significantly decreased in subcutaneous AT of pregnant women with PCOS compared non-PCOS pregnant women.
Conclusion: While previous studies showed that the expression of these genes didn't change in 2 groups, For the first time, we showed decreased expression of PIK3R1 and PIK3CA in AT of pregnant women suffering from PCOS. Decreased expression of PIK3R1 and PIK3CA can cause disruption in the metabolism of glucose, which may cause insulin resistance in pregnant women with PCOS which warrants further studies.
Keywords: Polycystic Ovary Syndrome, Subcutaneous Adipose Tissues, PIK3R1, PIK3CA

P-134: Identification of Potential Target Genes and Signaling Pathways of N-Acetylcysteine Related to Ovarian Cancer through Bioinformatics and Network Pharmacology Analysis

Khamineh Y*, Talkhabi M

Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
Email: m_talkhabi@sbu.ac.ir

Background: Ovarian cancer is a highly deadly form of gynecological cancer and it is crucial to use compounds that can control its development. N-acetylcysteine (NAC) is a promising chemopreventive agent with anticarcinogenic properties. This study aims to analyze the potential ovarian cancer target genes and signaling pathways of NAC using network pharmacology.
Materials and Methods: The GSE146553 dataset including OVCAR3 cell line and normal tissues were utilized from the Gene Expression Omnibus database (GEO) and additionally collected ovarian cancer-associated genes from the GeneCards database. Transcriptome analysis console (TAC) was used to identify differentially expressed genes (DEGs) that met specific screening criteria. The targets of NAC were obtained using the PharmMapper Server and SwissTarget Prediction, and common target genes were identified using Venn diagrams. Protein-protein interaction (PPI) networks of common targets were constructed using String and Cytoscape software. Finally, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted.
Results: This research, identified 108 potential targets of NAC and 2858 DEGs related to ovarian cancer. By analyzing the intersection of these two sets of genes, we identified 66 common potential targets that were involved in pathways related to lipid and atherosclerosis, progesterone-mediated oocyte maturation, and TNF signaling. Additionally, we identified 8 hub genes from the PPI network, including CASP3, HSP90AA1, MAPK1, MCL1, CCNA2, KIF11, TYMS, and KIT78 that had the highest degree.
Conclusion: Our study shows that NAC can be used in the treatment of ovarian cancer by targeting the genes involved in ovarian cancer, but further verification is needed to fully understand how it works.
Keywords: Bioinformatics, Network Pharmacology Analysis, N-Acetylcysteine, Ovarian Cancer

P-135: Methylation pattern of Immunological Genes Promoters in Recurrent Miscarriage Placenta Tissues

Khani H[1, 2*], Hossenpourefeizi MA[2], Safaralizadeh R[2], Mohseni J[1], Haghi M[2]

1. Genetic, ART Center of Tabriz ACECR, Tabriz, Iran

2. Genetic Department of Faculty of Science, Tabriz University, Tabriz, Iran
Email: jmohseni46@gmail.com

Background: Recurrent pregnancy loss (RPL) or recurrent miscarriage is a condition in which two or more consecutive miscarriages have occurred before the 20th week of pregnancy, which affects about 1-2% of all fertile women. Disturbance of the mother's immune system in rejecting the fetus/placenta is one of the most Effective factors affecting RPL, Because the fetus-pair is a semi-allograft with paternal and allogeneic antigens and any defect in the immunological tolerance process during pregnancy can lead to abortion. During pregnancy, multiple immune cells such as NK cells, Tregs, DCs, and macrophages reside in the decidua, which are crucial for the protection of the placenta. However, these cells can identify the placenta as a foreign immunological agent and cause it to be rejected by the mother's immune system. The HLA-G expressed on the extravillous trophoblasts (EVT) prevents their cytotoxicity through binding to the receptors on these cells. In addition, interleukin 10 (IL-10) is an anti-inflammatory cytokine that is secreted by some immune cells and trophoblasts, and inhibits the cytotoxicity of the mentioned cells through binding to IL-10R and activating the JAK1/STAT3 signaling pathway. Regarding to the importance of the function of immune molecules in RPL as well as methylation in the regulation of gene expression, the aim of this study is investigation of the methylation patterns of the HLA-G and IL-10R genes promoters in recurrent miscarriage placentas.
Materials and Methods: Placental tissues samples were collected from women with a history of at least two consecutive miscarriages. The Methylation Patterns of immune genes in placenta was evaluated by the MS-HRM methods.
Results: No significant difference was observed in HLA-G and IL10RB gene methylation status between recurrent abortion placenta samples and Normal placenta tissue samples.
Conclusion: It is likely that the regulation of the expression of the studied immune genes in the embryo placenta takes place through other mechanisms than DNA methylation, including miRNA, transcriptional factors and regulation of translation.
Keywords: Recurrent pregnancy loss, Placenta, MS-HRM, HLA-G, IL10R

P-136: Expression of SOCS3 Gene in Endometrium of Women with Endometriosis

Kia A[1*], Shahhoseini M[2], Favaedi R[2], Ghaffari F[3], Moini A[3, 4], Hosseini R[4], Amirchaghmaghi E[3]

1. Department of Biology, Faculty of Convergent Sciences and Technologies, Science and Research branch, Islamic Azad University, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Gynecology and Obstetrics, Arash Women's Health Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: amirchaghmaghi_e@yahoo.com

Background: Endometriosis is a common inflammatory disease in women in reproductive age characterized by the presence of endometrial tissue outside the uterus, mainly on perineum and pelvic organs. It is known immunological factors including cytokines are effective in the pathogenesis of endometriosis. Different cytokines signaling can lead to expansion of endometriotic lesions. The Janus kinase (JAK)- signal transducer and activator of transcription (STAT) signaling pathway plays a major role in cytokine receptor signaling. Dysregulation of this pathway by regulatory proteins like Suppressors of Cytokine Signaling (SOCS), Protein Inhibitors of Activated STATs (PIAS) and Protein Tyrosine Phosphatases (PTPs) are shown in various diseases. The aim of this study was to examine the gene expression of SOCS3, regulator of STAT3, in eutopic and ectopic endometrial tissues of women with endometriosis compared to normal endometrium
Materials and Methods: In this case-control study, two groups of women, endometriosis group(n=15) and control group (n=15) were enrolled after diagnostic laparoscopy. Ectopic endometrial samples were obtained through laparoscopic procedure while eutopic and normal endometrial tissues were obtained by pipelle. After total RNA extraction and cDNA synthesis, the expression level of SOCS3 gene was quantitatively determined by Real-Time PCR. Gene expression data were analyzed based on 2 -ΔΔct to estimate the relative fold change values.
Results: Gene expression of SOCS3 was detected in all studied endometrial samples. The finding of this study showed that the expression of SOCS3 gene was increased in the eutopic and ectopic tissues of women with endometriosis compared to the control endometrial samples but these increases were not significant. (P>0.05)
Conclusion: It seems that alteration in expression level of SOCS3 gene may be involved in pathogenesis of endometriosis. Further studies with larger sample size are needed
Keywords: Ectopic, Endometriosis, Eutopic, SOCS3

P-137: The Effect of Sildenafil Citrate on The Expression of The Anti-Apoptotic Genes During Sperm Cryopreservation in Infertile Asthenozoospermic Men

Kohzadi R[1*], Soleimani Mehranjani M[1], Cheraghi E[2], Shariatzadeh MA[1]

1. Department of Biology, Faculty of Science, Arak University, Arak, Iran
2. Department of Biology, Faculty of Science, University of Qom, Qom, Iran
Email: ronak.kohzadi@gmail.com

Background: Sperm cryopreservation, used in assisted reproductive technology is accompanied with many damages to the cell such as apoptosis. Adding antioxidant to the cryopreservation medium can ameliorate the freezing adverse effects. Sildenafil citrate (SC) has widespread applications and as an antioxidant, has many positive effects on sperm parameters. In this study the effects of SC on the expression of the anti-apoptotic genes in cryopreserved sperms of asthenozoospermic patients were investigated.
Materials and Methods: Thirty semen samples were collected from asthenozoospermic patients who had referred to the infertility treatment center of Qom University Jihad in 2021. Each sample was divided into 3 groups: Control (fresh); Freeze; and Freeze+ SC (cryo-protectant+ 0.67µM SC). Cryopreservation was performed through the rapid freezing method. In each sample, the expression of the genes Bcl-2 and HSP70 were ana-

lyzed using Real-time PCR. The results were reported as fold change (ΔΔCt formula). Data were analyzed statistically using Repeated Measure Analysis and Bonferroni post-hoc test.
Results: The expression levels of Bcl-2 and HSP70 genes increased significantly (2.64 and 2.85 times, respectively) in the Freeze group compared to the Control group (P=0.000), while the expression levels of these genes increased significantly in the Freeze+ SC group compared to the Control counterpart (4.15 and 2.89 times, respectively) (P=0.000).
Conclusion: Adding SC to the sperm cryoprotective medium, increases the expression of the genes Bcl-2 and HSP70, which could be considered as a compensatory response against apoptosis and oxidative stress induced by freezing.
Keywords: Anti-Apoptotic, Apoptosis, Asthenozoospermia, Cryopreservation, Sildenafil Citrate

P-138: Expression of vitamin D Receptor in the Endometrium of Women with Recurrent Implantation Failure After Vitamin D Supplementation

Kuroshli Z[1*], Ghaffari Novin M[1], Nazarian H[1], Abdollahifar MA[1], Zademodarres Sh[2], Shams Mofarahe Z[1]

1. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Clinical Research Development Center, Mahdiyeh Education Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: z_shams@sbmu.ac.ir

Background: Vitamin D receptor (VDR) binds to the vitamin D response element (VDRE) in the promoter of the target genes and it induces cell proliferation and differentiation. In early pregnancy, both decidua and trophoblast show an increase in the expression of the VDR gene which is associated with endometrial receptivity. The role of the vitamin D in the endometrium seems to be essential for the normal differentiation of decidual cells. Since the environmental factors are involved in the regulation of the VDR gene expression, we decided to investigate the effect of vitamin D supplementation on the VDR gene expression in the endometrium of women with recurrent implantation failure (RIF).
Materials and Methods: For this purpose, we selected twelve women with unknown RIF and vitamin D serum level deficiency (≤20 ng/ml) among the infertile women who had referred to Mahdieh infertility center from 2021 to 2022. After endometrial biopsy in the middle of the luteal phase, we checked the VDR gene expression by qRT-PCR technique. After that we prescribed the vitamin D supplement for three months. Endometrial biopsy in the same phase and the VDR gene expression check were done again.
Results: The results of statistical analysis showed the differences between the VDR gene expression before and after VD supplement prescription but they were not significant (P value<0.05).
Conclusion: Our findings did not show a significant increase in the VDR gene expression following vitamin D supplement prescription. This can be due to the small size of the studied group or their specific VDR gene polymorphism.
Keywords: Endometrium, RIF, VDR

P-139: The Effect of Vitamin D Deficiency on The Expression and Protein Levels of Prolactin, Insulin-like Growth Factor Binding Protein-1 and Homeobox Protein A10 in The Endometrium of Women with Recurrent Implantation Failure

Kuroshli Z[1, 3*], Ghaffari Novin M[2, 3], Nazarian H[2, 3], Abdollahifar MA[2, 3], Zademodarres Sh[4], Shams Mofarahe Z[2, 3,]

1. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Clinical Research Development Center, Mahdiyeh Education Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: z_shams@sbmu.ac.ir

Background: The main products of decidual stromal cells include prolactin (PRL), insulin-like growth factor binding protein-1 (IGFBP-1), as markers of decidualization, that can help to embryo implantation and maintenance of pregnancy. Furthermore, the expression of homeobox protein A10 (HOXA10) increases significantly in the middle of the luteal phase, and it interferes in the process of decidualization and embryo implantation. Forasmuch as Vitamin D (VD) regulates important genes for implantation and it is effective in maintaining pregnancy, we decided to evaluate the effect of vitamin D deficiency on the expression and protein levels of PRL, IGFBP-1 and HOXA10 in the endometrial cells by qRT-PCR and immunohistochemistry.
Materials and Methods: For this purpose, twelve women with history of unknown recurrent implantation failure (RIF) and vitamin D deficiency (≤20 ng/ml), and twelve women with unknown RIF and without vitamin D deficiency (≥30 ng/ml) from 2021 to 2022 were recruited into the study. In both groups, endometrial biopsy was performed in the middle of the luteal phase.
Results: Significant differences were shown in genes expression and protein levels of PRL, IGFBP-1 and HOXA10 in the endometrial cells between the two groups, especially in women with vitamin D ≥50 ng/ml compared to women with vitamin D ≤10 ng/ml (P value <0.05).
Conclusion: According to this study, it seems that vitamin D deficiency can contribute to disturbance in the process of decidualization and embryo implantation.
Keywords: Decidualization, Embryo Implantation, RIF, VD Deficiency

P-140: A Novel Analysis of ZBTB16 and DDX4 Expression in The Mice Seminiferous Tubules during Spermatogenesis

Mehdinezhad Roshan M[1*], Azizi H[2]

1. Department of Biology and Anatomical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Background: The spermatogonia cells (also known as Spermatogonial stem cell, SSC) which are localized along the basement membrane of the seminiferous tubules, starts one of the most important biological process during male lives, spermatogenesis, which finally results in the sperm cells production. In the present study we analyzed the co-expression of ZBTB16 and DDX4 in two types of cell populations present in seminiferous tubule.
Materials and Methods: This study was going to analyze the expression of the ZBTB16 and DDX4 in C57BL/6 mice by Im-

munohistochemistry (IHC) and STRING protein–protein interaction network based on reactome and KEGG pathways to reveal the direct linkage of genes involved in ZBTB16 and DDX4 during spermatogenesis.
Results: In this experimental study, whereas undifferentiated spermatogonial cells sharply express ZBTB16, other types of germ cells located in the seminefrous tubul were negative for this marker. In other hand, the germ cells near the basal membrane of seminefrous tubul showed expression of DDX4 wheras the undifferentiated germ cells located on the basal membrane were negative. Our protein-protein interaction analysis indicated that there is a tight interaction between ZBTB16 (zbtb16) and DDX4 (ddx4) with Klf4, Sox2, Nanog and Pou5f1 as most important factors for stem cell differentiation and Dazl, Gfra1, Tert, Vimentin and sox9 as other important factors during spermatogenesis. Further bioinformatic analysis clearly indicated crucial roles of both ZBTB16 and DDX4 in male reproduction systems including 'Male germ-line stem cell asymmetric division', 'Asymmetric stem cell division', 'DNA methylation involved in gamete generation', 'Male meiosis I', 'Cellular proc involved in reproduction in multicellular organism', 'Spermatogenesis', 'Male gamete generation' and 'Gamete generation' recpectively.
Conclusion: These results clearly proved the role of ZBTB16 as a specific marker for spermatogonial stem cells, and can be beneficial for advance research about *in vitro* differentiation of SSCs to functional sperms.
Keywords: DDX4, Germ Cells, Spermatogonial Stem Cells, ZBTB16

P-141: NGS Based PGT-A Importance in Recurrent Implantation Failure and Pregnancy Loss

Mohseni J*, Novroozi A, Khani H, Lotfivand A, Jafarzadeh S

Genetics, ART Center of East Azerbaijan ACECR, Tabriz, Iran
Email: jmohseni46@gmail.com

Background: Recurrent implantation failure and pregnancy loss are main challenge in reproductive medicine and Single cell pre-implantation genetics test for aneuploidy is one of the helpful capability for molecular karyotyping from embryo.
Materials and Methods: Cell lyzing and whole genome amplification from biopsied single cells of each embryo followed by NGS sequencing using thermo Fischer Reproseq kit and S5 Ion torrent sequencer. Chromosomal aneuploidy and or structural rearrangements analyzed after whole genome sequencing,
Results: More than 60% of embryos showed numerical and or structural rearrangements and were un-transferable. Normal transferable embryos showed successful immolation rate in previously RIF and RPL patient.
Conclusion: PGT-A is prefect technique in RIF, RPL and coupl's with chromosomal abnormality.
Keywords: PGT-A, PGT-SR, RIF, RPL

P-142: Association of BMP15 and FSHR Genes Variants with Primary Ovarian Insufficiency in Iranian Patients

Mousavi P[1, 2*], Masoudi NS[2], Almadani N[2], Mashayekhi M[3], Bazrgar M[1, 2]

1. Department of Molecular Genetics, School 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 Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: mbazrgar@royaninstitute.org

Background: Primary ovarian insufficiency (POI) is an important cause of female infertility, which is defined as the loss of ovarian function before age 40. Genetic factors already known to be responsible in 20-25% of POI cases. BMP15 and FSHR genes have an essential role in the maturation of follicles and the hormonal cycle. Some mutations of these genes are associated with POI. Those mutations of BMP15, exon 1, and FSHR, exon 10, which are associated with POI have been reported with frequencies <0.01 in Iranian general population according to Iranome, an Iranian genetic database of the general population. This study aimed to investigate association of variants located in the aforementioned exons with POI.
Materials and Methods: One hundred idiopathic POI cases were selected for genetic analysis. DNA was extracted from the peripheral blood of the patients. PCR-Sanger sequencing was performed in this case-series study to identify possible mutations in 100 POI patients. Chi-square test was performed to compare the genotype/allele frequencies between the POI patient and general population. American College of Medical Genetics and Genomics (ACMG) guideline and different bioinformatic databases were considered for prediction of consequences of the observed variants.
Results: The exonic rs41308602 (c.308A>G) and rs1557279925 (c.227G>A) were found in the BMP15 gene. The rs41308602 was detected in 14 cases (AA: 86%, AG: 13%, GG: 1%). Although its statistical difference with Iranome was marginal (P= 0.053), its association with POI has been previously reported. The rs1557279925 was found in one case (GG: 99%, GA:1%). There was no report of this variant in Iranome, however its allelic frequency in the studied cases was significantly higher than the highest reported of its minor allele frequency, P< 0.005. There is no clinical report of this variant but it was predicted as likely pathogenic. The exonic rs202162496 (c.1330G>A) and rs773201730 (c.1317G>A) were detected in FSHR analyses. The rs202162496 was found in one case (AA: 99% AG: 1%). Its frequency was not significantly different from Iranome (P<0.05) and was predicted as an uncertain variant; however, it has been reported in POI previously and sounds clinically important. The rs773201730 was detected in two cases (AA: 98% AG:2%) that was significantly higher than Iranome (P<0.05). There is no clinical report of it and has been predicted as likely benign to uncertain.
Conclusion: The rs41308602 and rs1557279925 of the BMP15 and the rs202162496 and rs733201730 of the FSHR, seems to be associated with POI, however association of the rs1557279925 has obviously certainty. Further investigation is recommended.
Keywords: BMP15, FSHR, Primary Ovarian Insufficiency

P-143: Altered Expression of MiRNAs in Sperm and Their Relationship with Oxidative Stress

Rahbar S*, Pashaiasl M, Ghaffari Novin M

Tabriz University of Medical Sciences, Stem Cell Research Center, Tabriz, Iran
Email: pashaim@tbzmed.ac.ir

Background: Approximately 15% of couples fail to conceive after one year of unprotected intercourse, with 50% of infertility cases attributed to the male factor. Oxidative stress is accepted to be the main contributing factor, that sperm quality and quantity affected.
Materials and Methods: To conduct this experiment, Ejaculates were collected from 55 infertile and fertile men provided. We studied the most common five spermatogenesis-related microRNAs expression levels in sperm. We also investigated the activity of antioxidant enzymes (superoxide dismutase SOD and glutathione peroxidase GPX) and production of malondialdehyde(MDA) and their relationship with miRNAs.
Results: According to our results, an increase in the activity of SOD, GPX and MDA production was observed in both severe oligoasthenoteratozoospermia (SOAT) and moderate oligoasthenoteratozoospermia (MOAT) sperm groups compared to the normal sperm group. In men with SOAT, the level of SOD production was significantly related to the expression of miR34c, miR184 and miR122. In men with MOAT, the level of GPX production had a significant negative correlation only with the expression of miR383. MDA production had a positive and significant relationship with miR34c and miR122 in MOAT and SOAT, and there was a significant negative relationship between miR383 and MDA in men with MOAT.
Conclusion: This increase in the amount of malondialdehyde and its relationship with microRNAs may help improve our understanding of male infertility and the strategies to prevent and treatment it.
Keywords: Antioxidant Enzymes, Male Infertility, Malondialdehyde, MicroRNA, Oligoasthenoteratozoospermia

P-144: Investigating The Interaction, and The Expression Of PTEN In Patients With Cryptorchidism, And Its Role In The Infertility

Reza E[1*], Azizi H[2]

1. Microbial Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
2. Nanobiotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Background: A gene called PTEN gives instructions for creating an enzyme that is present in practically all bodily tissues. The enzyme functions as a tumor suppressor, which implies that it aids in controlling cell division by preventing cells from proliferating (growing and dividing) too quickly or uncontrollably. Undescended testis (UDT), also known as cryptorchidism, is the result of the testes failing to descend into the scrotum during fetal development. It can be unilateral or bilateral. The risk factor for male infertility, cryptorchidism, has a significant influence on the architecture and histology of the testes, limiting reproductive function, and PTEN plays a crucial role in both conditions.
Materials and Methods: In this study, GSE25518 raw data was used, in which Twenty-three testicular biopsies from 22 boys were analysed (19 testes from 18 boys with cryptorchidism) and 4 contralateral descended testes from patients with testicular agenesis. Microarray testing was done after testicular biopsies were taken. Then Statistical Analysis, Interpretation of Microarray Data, and examining the Protein-Protein interaction network and the role of genes was performed using R/Bioconductor, String, Cytoscape, Gephi, and Enrichr.
Results: After the key genes with significant expression (adjusted P value<0.05, and log2-fold change>2) were identified, in the Protein-Protein interaction network, during the examination of Degree, Betweenness, Closeness, and Eigenvectors, it was found that the PTEN gene is the most key gene and has the main role in this gene network, which according to the analysis of the microarray results has a decrease in expression in cryptorchidism.
Conclusion: The findings and important functions of PTEN in the cell cycle and signalling pathways lead to the conclusion that this gene is one of the main factors contributing to infertility and may be utilized as a predictor of prognosis. Additionally, several novel approaches to personal treatment could concentrate on regulating PTEN expression.
Keywords: Azoospermia, Cryptorchidism, Male-Infertility Testicular-Diseases

P-145: Construction of CFTR Mutant Gene Model by Homologous Recombination System

Rezaee H[1*], Salehi M[1, 2], Bandepour M[1, 2], Kazemi B[1, 2]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University 6 of Medical Sciences, Tehran, Iran
2. , Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: h_rezaee101@yahoo.com

Background: The F508del in the CFTR gene is the most common mutation in cystic fibrosis (CF) disease which caused impaired protein folding, maturation, and transport to the surface of the cell, affecting the gastrointestinal and glandular as well as reproductive and respiratory systems. CF models are essential tools to understand and comprehensively investigate CF pathophysiology. In the present study, we aimed to design a transgenic CF model based on a homologous recombination (HR) system.
Materials and Methods: In this experimental study, a specifically designed construct containing the CFTR gene with F508del was cloned into a PTZ57R cloning vector and then the construct was transformed into the male pronucleus by microinjection after *in vitro* fertilization (IVF). Then the rates of blastocyst formation and embryonic development at 72 hours after IVF, were evaluated using microscope and PCR method.
Results: The CFTR gene was successfully cloned and overall, from 22 injected cells, 5 blastocysts were observed. PCR verification of the blastocyst with CFTR-specific primers represented complete recombination of CFTR into the mouse genome
Conclusion: For the first time we designed a unique construction that can be detected using a simple PCR method after the successful transforming of the construct into male pronucleus using microinjection and development of the zygote to the blastocyst stage. Production of CF mouse model by this specifically designed CFTR construct is recommended for further studies.
Keywords: Cystic Fibrosis Model, Homologous Recombination, IVF, PCR

P-146: Evaluation of Zona Pellucida Binding Protein 1 (ZPBP1) in Unexplained Infertile and Asthenoteratozoospermic Patients.

Rezaei Topraggaleh T[1, 2*], Rahimizadeh P[3], Numan Bucak M[4], Taravat M[5], Shahverdi AH[6]

1. Department of Anatomical Sciences, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2. Reproductive Health Research Center, Clinical Research Institute, Urmia University of Medical Sciences, Urmia, Iran
3. Cancer Research Program, The Research Institute of the McGill University Health Centre, Montreal, Canada
4. Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey
5. Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
6. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: shahverdi@royaninstitute.org

Background: Unexplained male infertility is a diagnosis reserved for men in whom semen analyses results are within normal values and physical as well as endocrine abnormalities were ruled out. This condition accounts for approximately 11% of male factor infertility cases. It is possible that the aberrant expression of zona pellucida binding protein 1 (ZPBP1) might be associated with fertilization failures in asthenoteratozoospermia and unexplained male infertility. ZPBP1 localizes to the acrosomal membrane and plays a role in binding to the oocyte zona pellucida. In our previous high-throughput protein analysis we found that this protein is down-regulated in asthenozoospermic samples. Therefore in this study we aimed to analyze the expression pattern of ZPBP1 in unexplained infertile and asthenoteratozoospermic semen samples.
Materials and Methods: Semen samples were obtained from unexplained infertile (n = 12) and asthenoteratozoospermic (n = 12) men who were referred to Royan Institute for infertility treatment after at least one failed or low fertilization in previous ICSI cycles. In addition, fertile controls (n = 12) who requested preimplantation gender selection for family balancing were recruited. Total protein of sperm samples were extracted using lysis buffer. Forty microgram of protein was loaded onto a discontinuous SDS PAGE. Then, the proteins were transferred to poly vinylidene difluoride membrane. Subsequently, after blocking membrane with nonfat dry milk, membranes were incubated overnight with primary antibodies against human ZPBP1. Finally by incubation of membrane with secondary antibody, the intensity of protein bands on the scanned X - ray films was quantified using the ImageJ software.
Results: Sperm morphology and motility was significantly lower in asthenoteratozoospermic group compared with fertile control and unexplained infertile patients. There were no statistically significant differences in sperm parameters between control and unexplained samples. The expression pattern of ZPBP1 was approximately 3 fold decreased in asthenoteratozoospermia compared to other groups. While no significant differences were seen between the fertile control and unexplained infertile patients.
Conclusion: Our result showed that, expression pattern of ZPBP1 is more related to morphology of spermatozoa because in asthenoteratozoospermic samples significant reduction of protein is seen. Moreover, unexplained infertile men contained normal expression of ZPBP1 proteins.
Keywords: Asthenoteratozoospermia, Unexplained Male Infertility, Zona Pellucida Binding Protein 1

P-147: Association OF Sohlh1 Gene Variants with Iranian Primary Ovarian Insufficiency Patterns

Safari A[1, 2*], Daneshipour A[2], Mashayekhy M[3], Almadani N[2], Bazrgar M[2]

1. Department of Molecular Genetics, School 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 Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: mbazrgar@royaninstitute.org

Background: Primary ovarian insufficiency (POI) is one of the main causes of amenorrhea and primary and secondary infertility. POI causes premature infertility in women under 40 years of age. A number of genes involved in it are known, one of them is SOHLH1 with known role in early stages of ovulation. In this research, the association of exon 4 variants of SOHLH1 and its related splicing regions with POI was investigated.
Materials and Methods: PCR-Sanger sequencing was performed in this case-series study to identify variants in 100 POI patients for the desired region. Statistical analysis was performed with Chi-square and Fisher's exact test methods to compare genotypic and allelic frequencies, with significance level less than 0.05. Iranome and global genetic databases were used to compare frequencies with general population. American College of Medical Genetics and Genomics (ACMG) guidelines and different bioinformatic databases were considered for prediction of consequences of the observed variants.
Results: In the studied region, 5 variants were found. The rs7032532 intronic variant, was found in 43 patients, 33 heterozygous and 10 homozygous. The allelic and genotypic frequencies of this variant were significant compared to Iranome. The rs503539 and rs503511 intronic variants were observed in 43 patients, all homozygous. These 3 frequent intronic variants were predicted as benign to likely benign. The rs140132974 splice acceptor variant of uncertain significance was found in one patient. Its allelic and genotypic frequency was not significantly different from Iranome. A novel stop-gain homozygous variant was found in two patients and after double confirmation by Sanger sequencing was registered in the Clinvar database as likely pathogenic. It has a completely destructive effect due to premature termination of transcription in exon 4 instead of exon 11 and production of truncated protein.
Conclusion: The rs140132974 and the novel nonsense mutations sound to be associated with POI and clinically significant. Further investigation is recommended.
Keywords: Primary Ovarian Insufficiency, POI, Rs140132974, Rs7036532, SOHLH1 Gene

P-148: Reduced Expression of The E-cadherin Adhesion Molecule in The Fallopian Tubes of Diabetic Mothers

Shavandi M*, Foroutan T, Nikkhah ZS

Department of Animal Sciences, Kharazmi University of Biological Sciences, Tehran, Iran
Email: tataforoutan@gmail.com

Background: Glucose intolerance with varying severity starts or is diagnosed for the first time during pregnancy is called gestational diabetes. Diabetes during pregnancy has many effects

on the mother and fetus. Maternal gestational diabetes stimulates insulin secretion and hyperplasia of pancreatic β cells, macrosomia, increase in basal metabolism, increase in oxygen consumption and even fetal hypoxia. Since the oocytes released from the ovary pass through the fallopian tube, the adhesiveness of the lining cells of this area may change in diabetic mothers. In the present research, we aimed to compare the expression level of E-cadherin adhesion molecule in the fallopian tube of diabetic mothers with healthy mothers.

Materials and Methods: In the current experimental study, female rats(180-200g) were divided into two diabetic and control groups(n=8). To induce diabetes, intraperitoneal injection of streptozocin was used in female rats. The animals of both groups were caged with male mice of the same breed for one night. If vaginal plague was observed, the next morning was considered as the first day of pregnancy. On the third and fourth days of pregnancy, the mice were anesthetized by injecting two substances, ketamine and xylene, and blood was taken from the heart. After centrifuging the blood at 2500 rpm for 5 minutes, the resulting serum was separated from the body for tissue preparation and then H&E and immunohistochemical staining for E-cadherin protein

Results: The results of this study showed that the expression of the adhesion molecule E-cadherin in the fallopian tubes of diabetic mothers is lower than healthy mothers. Immunohistochemical analysis showed that in addition to the decrease in the expression of the E-cadherin adhesion molecule in fallopian tube of diabetic mothers, there was an increase in tissue secretions in this area.

Conclusion: According to the results of the present study, it can be concluded that there is a direct relationship between mothers suffering from diabetes, the complications caused by diabetes in offspring and the decrease in the expression of adhesion molecules in the fallopian tubes.

Keywords: Diabetes, Diabetic Mothers , E-cadherin, Fallopian Tube, Pregnancy

P-149: The Expression of MiRNA-761 and MiRNA-3619 Are Increased in Granulosa Cells and Blood Serum in Women with Diminished Ovarian Reserve (DOR)

Tafazzoli P[1*], Siadat F[2], Mashayekhi M[3], Motahari Rad H[1], Fathi R[1]

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

2. Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran

3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Email: rfathi79@royaninstitute.org

Background: miRNAs are short sequences of 17 to 20 nucleotides that play a role in all physiological, pathological and biological processes. All body cell processes such as division, growth and differentiation, apoptosis and necrosis, etc. are under their control. Also, if the expression changes are recognized, they may be used in direction of diagnosis, treatment, and even prediction of the disease.

Materials and Methods: In this basic study, 10 people in two categories of DOR and control group were investigated and miRNA-761 and miRNA-3619 expression changes were evaluated in their granulosa cells and blood serum samples. The age of the participants was under 30 and their BMI was between 25 and 30 cm. The expression changes of miRNAs were evaluated by Q-PCR and compared with the T-Test program of Prism software.

Results: miRNA-761 and miRNA-3619 showed significant increase in the DOR group compared to the control group. This amount of increased expression was observed in both granulosa cells and blood serum samples of the participants.

Conclusion: Since the changes of miRNAs expression in both blood serum and granulosa cells had almost the same pattern and showed an increase in expression compared to the control group, this novel data is the first step to introduce both miRNA-761 and miRNA-3619 as specific biomarkers for prediction of DOR and premature ovarian failure (POF) diseases.

Keywords: Bioinformatics, POI, DOR, MiRNA

P-150: Down-Regulation of Long Non-Coding HOX 11Transcript Antisense Intergenic RNA(HOTAIR) following Laparoscopic Salpingectomy Is Associated with Reduction of Local Endometrial Inflammatory Response in Patients with Hydrosalpinx

Zafarani F[1, 2*], Soleimani Mehranjani M[1], Ghaffari F[3], Shahhoseini M[4], Shariatzadeh SMA[1], Lotfi M[5], Piryaei A[6]

1. Department of Biology, Faculty of Science, Arak University, Arak, Iran

2. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

3. Department of Reproductive Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

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

5. Department of Pathology, Amir AalamHospital, Tehran University of Medical Sciences, Tehran, Iran

6. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email: fzafarani1391@gmail.com

Background: Ten infertile women with moderate to severe hydrosalpinx who underwent laparoscopic salpingectomy, were recruited–luteal-phase endometrial samplings were performed at the time of surgery and in forth-post treatment cycle. Ten fertile women who have had successful pregnancy were considered as control. RNA extraction and cDNA synthesis were done and real-time PCR technique was used for quantitative HOTAIR gene expression. For evaluation of local endometrial inflammatory response, the leukocytes population count was recorded in five representatives adjacent and nonoverlapping high-power fields (400 X) for pre and post salpingectomy endometrium.Wilcoxon signed Ranks Test and kruskal-Wallis were used for data analysis. P-value less than 0.05 was statistically significant.

Materials and Methods: Ten infertile women with moderate to severe hydrosalpinx who underwent laparoscopic salpingectomy, were recruited–luteal-phase endometrial samplings were performed at the time of surgery and in forth-post treatment cycle. Ten fertile women who have had successful pregnancy were considered as control. RNA extraction and cDNA synthesis were done and real-time PCR technique was used for quan-

titative HOTAIR gene expression. For evaluation of local endometrial inflammatory response, the leukocytes population count was recorded in five representatives adjacent and nonoverlapping high-power fields (400 X) for pre and post salpingectomy endometrium. Wilcoxon signed Ranks Test and kruskal-Wallis were used for data analysis. P-value less than 0.05 was statistically significant.
Results: Expression levels of HOTAIR significantly decreased following salpingectomy (P=0.01). Moreover, a significant decrease in the number of lymphocytes was observed post surgery (P=0.017). There were no significant change in the number of neutrophils, plasma cells, eosinophils, or macrophages post surgery (P>0.05).
Conclusion: Salpingectomy down-regulates HOTAIR expression, accompanied with reduced endometrium local inflammatory response.
Keywords: Endometrial Inflammatory Response, HOTAIR, Hydrosalpinx, Salpingectomy

P-151: Association of -308G/A TNFα Gene Polymorphism with Reduced Risk of Idiopathic Infertility in Men

Zamani-Badi T[1*], Nikzad H[2]

1. Gametogenesis Research Center, Institute for Basic Sciences, Kashan University of Medical Science, Kashan, Iran
2. Anatomical Sciences Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran
Email: nikzad_h@kaums.ac.ir

Background: Infertility is one of the major health problems in the world and several factors play a role in the occurrence of this complication. One of the causes of idiopathic male infertility is a defect in the process of spermatogenesis due to genetic changes in cytokines involved in this process. Tumor necrosis factor-alpha (TNFα) as a multifunctional cytokine controls spermatogenesis-related cellular activity. In this study, the association of -308G/A polymorphism in the TNFα gene with male infertility was investigated.
Materials and Methods: In a case-control study, blood samples were collected from 82 infertile men and 107 fertile men. After DNA extraction, the genotype of the samples at the -308G/A region was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.
Results: Data analysis showed a significant association between GA genotype and reduced risk of male infertility. Also, in the subgroup study, a significant association was observed between this genotype and the reduction of oligozoospermia and asthenozoospermia risk. Similar results were found for the association of carriers of allele A (GA + AA) and idiopathic male infertility. In addition, the allelic analysis showed a significant association between allele and a reduced risk of idiopathic male infertility. Subgroup analysis showed a significant association between this allele and reduced risk of asthenozoospermia, also.
Conclusion: Based on findings of this research, the TNFα -308G/A polymorphism can be considered as a protective factor and a potential biomarker for idiopathic male infertility.
Keywords: Cytokine, Male Infertility, -308G/A Polymorphism, TNFα Gene,

P-152: LncRNA DANCR and CASP10 Might Be Involved in Apoptosis of Cycling and Differentiating Spermatogenetic Cells Leading to Male Subfertility

Zandieh Z[1*], Mozdarani H[2], Shahhoseini M[3]

1. Department of Andrology, Royan Institute for Reproductive Biomedicine, Tehran, Iran
2. Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Genetics, Royan Institute for Reproductive Biomedicine, Tehran, Iran
Email: mozdarah@modares.ac.ir

Background: Infertility is usually defined as the inability of a couple to conceive even after one year of unprotected, frequent sexual intercourse. One of the common causes of male infertility is the decrease in the number of sperm cells and the low motility of sperm cells in men. LncRNA DANCR was shown to be involved in many regulatory signaling pathways and can play a role in various cellular processes such as cancer cell proliferation, cell differentiation, cell cycle and apoptosis through multiple regulatory functions such as chromatin regulation, transcriptional regulation, RNA modification, etc. .CASP10 encodes a protein that is a member of the caspase family and plays a role as an initiator caspase in the process of apoptosis. The purpose of this study was to evaluate the expression level of LncRNA DANCR and CASP10 in mature sperms of subfertile patients.
Materials and Methods: To do this, 10 healthy control, 10 Oligoteratozoospermia patients, 10 Asthenoteratozoospermia patients and 10 Oligoasthenoteratozoospermia patients were included in the study. RNA extraction, cDNA synthesis was performed according to the manufacturer's instruction. Real-time PCR was performed for all samples and data were evaluated using 2-ΔΔCt method.
Results: Results might be indicative that CASP10 play a role in apoptosis leading to reduced number of sperms in different spermatogenic cycles. While LncRNA DANCR might not be involved in different cycles of spermatogenesis and needs further investigation.
Conclusion: Data showed that the expression of CASP10 increased with a p.value <0.05 in Oligoteratozoospermia patients compared to normal individuals and LncRNA DANCR was not significantly different among infertile men compared to normal individuals
Keywords: Asthenteratozoospermia, CASP10, LncRNA DANCR, Male Infertility, Oligoteratozoospermia,

P-153: The comparing Antioxidant Effects of Hypotaurine and Melatonin on Human Sperm Classical Parameters, Acrosome reaction, DNA fragmentation, and Expression Level of HspA2 and Caspase 9 During Rapid Freezing

Zolfagharizadeh A[1*], Rashki L[2], Esmaeili V[2] , Alizadeh A[2, 3], Rezazadeh Valojerdi M[4], Shahverdi A[2]

1. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute, Tehran, Iran
3. Gyn-medicum, Center for Reproductive Medicine, Göttingen, Germany
4. Department of Anatomy, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
Email: shahverdi@royaninstitue.org

Background: One of the discussed approaches for dealing with cryoinjuries during human sperm cryopreservation is the use of antioxidants. This study compared the effects of melatonin (MEL), an intracellular antioxidant, and hypotaurine (HYP), an extracellular antioxidant, on the routine and functional test of sperm, and the expression of HspA2 and Caspase9 during rapid freezing of human sperm.
Materials and Methods: After obtaining 34 normospermia semen samples, each sample was divided into four experimental groups: fresh, control freezing (which include human tubal fluid medium and 0.5M sucrose), and two freezing groups that were treated with 50 mM HYP and 2 mM MEL, respectively. To perform rapid freezing, a volume of 200 µl of the sample was transferred into a straw and subsequently cryopreserved in liquid nitrogen. The expression levels of HspA2 and Caspase9, along with the sperm classical parameters, viability, acrosome integrity, and DFI, were evaluated in both pre-and post-rapid freezing.
Results: As expected, the fresh group exhibited a significantly higher percentage of sperm classical parameters, viability, and acrosome integrity (P<0.05), as well as a lower level of DFI (P<0.05) in comparison to the freezing groups. In freezing groups, the HYP group showed an upper percentage of motility, morphology, and viability compared to others freezing groups (P<0.05). Additionally, the results indicated that the HYP group exhibited significantly lower levels of DFI, and acrosome reaction compared to the control freezing and MEL groups (P<0.05). However, the expression level of HspA2 was significantly higher in the group that received MEL treatment (P<0.05). The expression of Caspase 9 was not changed by the addition of MEL and HYP.
Conclusion: While the intracellular antioxidant MEL increased the expression of HspA2, the extracellular antioxidant HYP displayed a greater protective effect on sperm classical parameters, acrosome integrity, and DFI during rapid freezing.
Keywords: Human Sperm Cryopreservation, Hypotaurine, Melatonin

Imaging

P-154: To Compare The Success Rate of Assisted Reproductive Technology in T-Shaped and Normal Uterus

Ahmadi F*, Pahlavan F, Niknejad F, Irani Sh

Department of Reproductive Imaging, , Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, , Tehran, Iran
Email: dr.ahmadi1390@gmail.com

Background: According to the ESHRE classification T_ Shaped uterus belongs to the Class U1: Dysmorphic uterus. Hysterosalpingography (HSG) and Ultrasound are used to diagnose of T_Shaped uterus. The prevalence of T-shaped uterus is reported about 0.02 -5%. It is said that T-Shaped uterus could relate to poor reproductive outcomes. We aim to find the success rate of ART in T-Shaped uterus and normal group.
Materials and Methods: A cross-sectional study was done in Royan Institute between 2019-2020. Information was extracted from patients' files. According to the inclusion criteria, patients were selected and categorized to T-shaped and normal groups. HSG was done for all infertile patients, and T-shaped uterus were approved by hysterosalpingography. We compared the success rate of IVF in both groups.
Results: Cases that were defined as medium were omitted. Total sample size was 468. The frequency of the T-shaped uterus was 19.4% and the frequency of the normal uterus was 80.6%. Then the positive chemical pregnancy was calculated that was 47%. Positive pregnancy in the T-Shaped group and normal group was 42.8% and 48.01%, respectively. The difference was not significant (P value>0.05). Our finding reminds that metroplasty should not be a routine treatment for T-Shaped uteruses. It should be considered in RIF cases, recurrent abortions, and those who are symptomatic.
Conclusion: There was no significant difference between the outcomes of ART in the T-Shaped group and normal group. More studies are necessary to find the efficacy and the need of medical interventions in T-shaped uteruses.
Keywords: ART, Success Rate, T-shaped,

P-155: Early Pregnancy in Hysterosalpingography

Ahmadi F*, Hosseini F , Pahlavan F
Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: dr.ahmadi1390@gmail.com

Background: Hysterosalpingography (HSG) remains a reliable technique in assessing luminal patency of the fallopian tubes and the shape of uterine cavity in spite of all advanced diagnostic methods. In respect of the increasing the average age of first-time mothers and progresses in assistive reproductive medicine, the number of hysterosalpingography requests has substantially raised. Thence the likelihood of patients presenting with unsuspected early pregnancies prior to HSG has increased as well. Despite using pregnancy test and accurate menstrual history a few numbers of unexpected pregnancy reports have been still published. For women with a positive history of irregular menstrual bleeding or amenorrhea, both mid-cycle bleeds or early pregnancy bleeds could be misinterpreted as regular menses. Hereupon this test is susceptible to error and may be performed accidentally on pregnant women.
Materials and Methods: This study is based on the review of the hysterograms performed at the imaging department of Royan Institute.
Results: Early pregnancy can be recognized by some radiological features as following; double-outline uterine cavity (DOUC), intrauterine filling defect and irregularity with intravasation. Uterine cavity enlargement and elongation may be visualized as well. DOUC is the earliest specific sign of pregnancy which is seen as a thin line of water-soluble contrast medium surrounds the uterine wall. Filling defect is the other sign that is caused by the intrauterine gestational sac. Ectopic pregnancies following HSG is the other condition that has reported in hysterosalpingography and may result in tubal damage or possibly a 'flushing effect' of the amniotic sac by the contrast media.
Conclusion: It is significant to recognize early pregnancy signs for hysterographer to terminate the introduction of contrast medium. Furthermore, the differentiation of these signs from other anomalies or disorders has the notable role in interpretation.
Keywords: Early Pregnancy, Hysterosalpingography, Unsuspected Pregnancy

P-156: The Frequency of Coexistence of Polyp and Fibroma in the Uterus of Infertile Women Referred to The Royan Institute

Niknejadi M*, Pahlavan F, Irani Sh , Niknejad F

Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: mniknezhady@gmail.com

Background: Infertile women, especially those with recurrent miscarriages, should be evaluated for uterine abnormalities. Acquired lesions that include uterine adhesions, polyps and fibroids cause changes in the shape of the uterine cavity. Sometimes polyp and fibroma are seen together. The current study was designed to determine the frequency of coexistence of endometrial polyps and fibroids in infertile patients referred to Royan Institute.
Materials and Methods: This study was a cross-sectional study. The statistical population includes 123 infertile women who meet the inclusion criteria, and have been referred to Royan Research Institute for infertility treatment from October 2020 to the end of October 2021. Patients who underwent hysteroscopy to investigate fibroma or polyp anomalies were selected. SPSS software was used to perform descriptions.
Results: The patients' age ranges between 27 and 45 year. (Mean=37.9 ±4.1). Out of 123 patients, 87% had primary infertility and 13% had secondary infertility. Using ultrasound examination, different types of fibroids, including 59 submucosal fibroids, 53 intramural fibroids, and 11 subserosal fibroids were detected. Out of these 123 cases with fibromas, 71 patients (57%) had polyp. In the final examination by hysteroscopy, out of a total of 123 patients, 47 cases (38.2%) had fibroids with pressure impact, and only 12 cases (9.8%) had polyps, confirmed by pathologic assessment.
Conclusion: Considering the simultaneous occurrence of polyps and fibroma, and their common pathophysiology, if one of these anomalies is observed, the occurrence of the other should be checked. The final decision should be made by hysteroscopy and pathology.
Keywords: Coexistence, Fibroma, Polyp

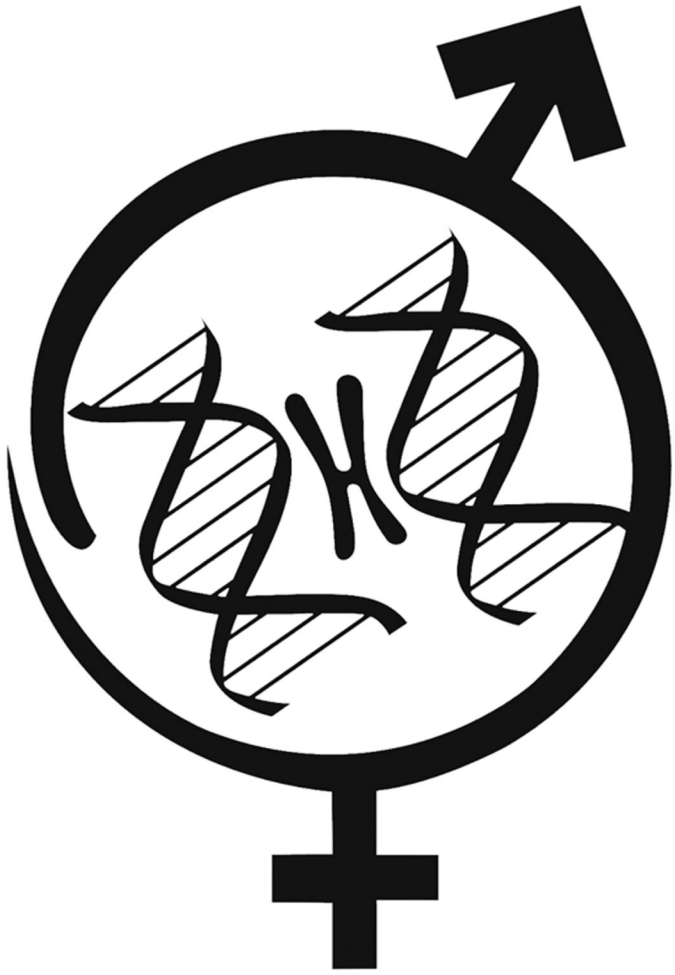
Authors Index

Abstracts of

Royan International Hybrid Twin Congress

18th Seminar on Nursing and Midwifery

31 August 2023



Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Invited Speaker

Inm-1: Role of Exercise in POF Complications Improvement

Afghan M

Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: mar.afgh@gmail.com

Apart from menopausal symptoms (e.g., hot flushes, urogynecologic and sexual changes), there is increased risk of cardiovascular, musculoskeletal, neurocognitive, and metabolic dysfunction, and overall increased mortality. Hypoestrogenemia is associated with multiple adverse effects on body composition, including accelerated bone loss, muscle decline, and augmented visceral fat deposition.
Although the pathogenesis of POI is diverse, these disorders share the common variable of hypoestrogenism that may result in decreased bone accrual, failure to attain peak bone mass, as well as increased bone loss leading to an overall decline in bone health. POI is associated with compromised bone health regardless of its etiology. Increased fracture risk of up to 1.5 times has been reported in patients with POI, compared with women experiencing menopause at a normal age. …… supervised protocols demonstrate significantly higher effects on dynamic balance, strength, and power, i.e., parameters related to fall risk and bone strength. the superiority of supervised (resistance) exercise programs might be related to higher adherence, motivation, intensity progression, and safety. Exercise programs on bone strengthening applied intensive resistance, weight bearing, and impact exercises.
Healthy women experience a slow decline in muscle mass averaging 0.4 to 0.8 kg per decadestarting at the age of 20, but this process is accelerated after menopause implicating estrogen deficiency. Menopause and aging are associated with a more significant decline in strength than muscle mass; however, both components play an essential role in regulating muscle health.
Skeletal muscle fibers are broadly categorized as “slow- twitch” (type 1) and “fast-twitch” (type 2) fibers. These coexist in a single muscle group in varying proportions depending on the muscle activity. Menopause and aging are associated with the loss of type II fibers, which may explain the slow responses in older individuals. Moreover, skeletal muscle has a higher proportion of ER-α on type II fibers, and it is postulated that estrogen has a protective influence/effect on muscle power through its action on type II muscle fibers.
Physical exercise is pivotal for the maintenance of muscle well-being. Exercise exerts its beneficial effects in the muscle at molecular and cellular levels via activation of the mammalian target of rapamycin pathway, promoting mitochondrial protein synthesis and reducing reactive oxygen species concentration. Furthermore, exercise modulates differentiation of satellite cells into myoblasts instead of adipocytes, resulting in enhanced muscle volume and reduced fat infiltration. Exercise therapy can be grouped into endurance training (aerobic), resistance training, and combined training. Endurance training boosts functional capabilities, while resistance training helps in building muscle volume and improving its quality. Combined training integrates advantages of both modalities resulting in improved muscle strength and endurance and BMD. Balance and functional training substantially reduce falls and fractures in older population but can also be useful in improving musculoskeletal health in POI group.
Whole-body vibration therapy is a training method that increases exercise efficiency by transmitting loads in a precise and short time by adjusting the amplitude and frequency of vibrations and showed significant increases in muscle strength and hypertrophy in the whole-body vibration resistance training group as compared with resistance training group only.
Lifestyle modifications, such as regular physical exercise, especially aerobic exercise, are also often recommended as main or adjuvant therapy to minimize cardiovascular risks and general symptoms of reduced ovarian function.

Inm-2: Obstetrics Complications in Pregnancy

Ahmadi M

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: Maryam.ahmadi11395@gmail.com

Pregnant people with obesity are at increased risk for an array of maternal and perinatal complications, and the risks are amplified with increasing severity of the condition .
It has been estimated that one-quarter of pregnancy complications [(eg, gestational hypertension, preeclampsia, gestational diabetes, preterm birth, large for gestational age (LGA) infant] are attributable to maternal obesity or overweight.
Patients with prepregnancy obesity followed by high gestational weight gain have the highest risks of pregnancy complications.
Offspring of pregnant people with obesity are at increased risk of developing obesity in childhood and as adults.
Obstetric providers should be aware of these risks and modify patient care before pregnancy, during pregnancy, and postpartum to reduce the risk of these adverse outcomes.
Although clinical practice guidelines for management of pregnant people and people planning pregnancy with obesity vary, they consistently recommend pregnancy risk counseling, a healthy diet, exercise, and dietician referral for managing weight loss and gestational weight gain.
This standard definition/classification for the nonpregnant population do not adapt well to the pregnant population since a pregnant person's weight increases over a relatively short interval of time, and much of the weight gain is related to accretion of mass (ie, fetus, placenta, amniotic fluid, blood) that will be lost at delivery. Since no standard pregnancy-specific definition of obesity exists, pregnant patients are often considered obese or nonobese based on their prepregnancy body mass index.

Inm-3: Infertile Men: Their Sexual Function; Identity and Relationships

Azin SA

Social Medicine, Avicenna Research Institute, ACECR, Tehran, Iran
Email: saliazin@gmail.com

One out of every 4 to 5 Iranian couples experience primary infertility. During the past few decades, several researches have been conducted regarding the effect of facing infertility on the sexual health of couples. Although the results of these studies have been somewhat contradictory, these studies show that infertility has had a significant impact on couples' sexual

relations. Apparently, in terms of culture as well as therapeutic approaches, women are facing more pressure in this regard. However, this does not mean that men do not experience sexual problems in this situation. Various aspects of men's sexuality can be affected after infertility diagnosis. All three functions of sexual desire, sexual arousal and orgasm can be disturbed in infertile men. Infertile men can also have problems with their sexual identity and sexual self-steam; Especially in patriarchal societies where having children and the possibility of continuing the generation is considered very important. At the same time, infertility affects the overall relationship and sexual relations of couples. Some of the sexual problems of infertile men show up with the start of treatment interventions. One of the most important problems is sexual dysfunction due to a condition called "sex on demand".

Therefore, screening for sexual problems in infertile couples and providing services to them should be an integral part of the infertility treatment centers.

Keywords: Infertility, Infertility Treatment, Sexual Identity, Sexual Function, Sexual Health

Inm-4: What Are The Characteristics of A Well-Balanced Diet for Controlling Gestational Diabetes?

Eslamian G

Department of Cellular and Molecular Nutrition, Faculty of Nutrition and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email: gh.eslamian@sbmu.ac.ir

Gestational diabetes mellitus (GDM) is defined as the development of glucose intolerance during pregnancy. This condition carries various risks for both mother and baby, including birth complications, the birth of larger-than-normal babies, and an increased risk of type 2 diabetes in both. Making lifestyle changes is crucial for managing GDM, and the first line of treatment involves medical nutrition therapy, along with weight management and physical activity. In this article, we aim to provide an overview of the most important dietary interventions and components for treating and guiding women with GDM throughout their pregnancy. It is essential for all women with GDM to receive dietary advice from a clinical dietitian, as this is a fundamental aspect of treatment. The nutrition plan should be continuously adjusted based on factors such as self-monitored glucose levels, appetite, weight gain patterns, as well as the woman's dietary preferences and daily activities. If insulin therapy is necessary, maintaining consistent carbohydrate intake during meals and snacks becomes a primary goal to aid in insulin adjustment. The woman should be given guidance on how to create a varied diet and avoid high post-meal blood glucose levels. Paying close attention to carbohydrate intake is particularly important, as the type, amount, and distribution of carbohydrates significantly impact blood sugar levels after eating. Although some studies have shown a connection between certain nutrients or dietary patterns and the prevalence of GDM, further research is needed to evaluate the effects of supplements in larger trials involving a more extensive patient population.

Inm-5: Familiarity with Treatment Issues in Infertility Centers

Ezabadi Z[1, 2]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Email: Z_ezabadi@yahoo.com

Patient centredness is one of the six dimensions of quality of care according to the 'Institute of Medicine' in the USA. However, the consensus on the most appropriate international conceptualization of patient-centred care (PCC) is lacking. Several definitions and models for PCC, allied concepts (e.g.relationship-centred care) and components of PCC have been proposed. However, only few models for the concept of PCC were actually based on the patients' perspective. Instead, most were based on the perspective of professionals.

The patient-centredness of infertility care depends on 10 detailed dimensions, which can be divided into system and human factors, and there is a two-way interaction between both kinds of factors. System factors, in order of patient's priority, are: provision of information, competence of clinic and staff, coordination and integration, accessibility, continuity and transition and physical comfort. Human factors, in order of patient's priority, are: attitude of and relationship with staff, communication, patient involvement and privacy and emotional support.

This presentation provides a detailed patient's description of the concept 'patient-centred infertility care' and an interaction model that aids understanding of the concept. Fertility clinics are encouraged to improve the patient-centredness of their care by taking into account the detailed description of the dimensions of patient-centred infertility care, and by paying attention to both system and human factors and their interaction when setting up 'patient-centred improvement projects.

Inm-6: Nutrition Intervention for Improving Pregnancy Complications and Outcome: Supplementation Vs Dietary Interventions

Giahi L

Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Email: drlgiahi@gmail.com

There is substantial evidence on the importance and complex relationship of maternal diet for the health of the fetus as well as short term and long term 0ut comes of birth. This golden period of health engineering for next generation is some how influenced by supplement industry and dietary interventions are mainly focused on supplementations. Also, expectant mothers normally focus on their health and nutrient intake more than any time, therefore supplement use is very common in pregnancy and even more emphasized than proper meal planning from health care systems.

Given the relatively high incidence of nutritional deficiencies, reduced nutrient density of food, and higher intake of nutritionally poor processed food, supplement use in pregnancy may be beneficial in some instances and reduce risks of negative outcomes such as preeclampsia, GDM, and SGA. However, it is worth noting that significant limitations exist for assessing the correct level of nutritional recommendation.

Nutrient deficiencies may also develop over time, which are not always resolved during a short window of supplementation as beginning supplements in the first or second trimester may not

be a sufficient time frame in which to affect development of the fetus. Therefore, nutrition assessment and nutrition screening must be recommended as early as couples are planning for a baby specially in high-risk couples.
Proper management of conditions such as GDM, SGA are needed to be addressed by nutritional intervention in obese, overweight an PCOs mothers with balanced macronutrient and micronutrient distribution day by day from early pregnancy.
Adverse nutritional status in pregnant mothers is needed to be tracked to provide recommendations fitting to individual's needs. Ways to recommend a diet that meets eating habit dynamics must be planned according to the reality of each socioeconomic condition and health care system.

Inm-7: The Primary Ovarian Insufficiency and Its Effect On Life Heath of Women Major Complications of Primary Ovarian Insufficiency

Mashayekhi M

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: ???

Infertility: Inability to get pregnant can be a complication of primary ovarian insufficiency. In rare cases, pregnancy is possible until the eggs are depleted.
Osteoporosis: The hormone estrogen helps maintain strong bones. Women with low levels of estrogen have an increased risk of developing weak and brittle bones (osteoporosis), which are more likely to break than healthy bones.
Depression or anxiety. The risk of infertility and other complications arising from low estrogen levels causes some women to become depressed or anxious.
Heart disease. Early loss of estrogen might increase your risk.
Primary ovarian insufficiency is usually permanent. Most people with the condition take long-term hormone therapy to manage symptoms and reduce the risk of complications. Primary ovarian insufficiency causes low estrogen levels. Losing estrogen can have side effects similar to those that occur with menopause such as hot flashes, decreased sex drive, mood changes, and neurological effects that overall reduced life expectancy.
Infertility: Premature ovarian failure causes menstrual disorders, even complete amenorrhea, and leads to infertility in women; Osteoporosis: The hormone estrogen plays a role in keeping bones strong in women. The decrease in estrogen levels in postmenopausal women or women with premature ovarian failure will lead to osteoporosis, bone and joint pain; bone fractures Anxiety, depression: Concerns about health, beauty, pregnancy, plus the impact of the drop in estrogen hormone can make ovarian failure patients fall into a state of crisis, anxiety and fear. than the risk of depression. Primary ovarian insufficiency often causes feelings of sadness and loss, especially if they still had hopes for getting pregnant. Psychosocial stress caused by stressful life event , has been associated with increased susceptibility to many diseases, such as cardiovascular disease, neurodegenerative disorders, and cancers.
Cardiovascular disease: Although very rare in young women with premature ovarian failure, if combined with some other bad factors, it can increase the risk of developing cardiovascular disease later in life; POI include the following: cardiovascular (endothelial dysfunction, ischemic heart disease, myocardial infarction, etc.), Endocrine disease: Hypothyroidism: The thyroid gland controls the body's metabolism. Decreased thyroid hormone will affect metabolism and affect many organs in the body, mental decline and physical decline. Another endocrine disorders are: metabolic syndrome, diabetes mellitus.
Urogenital disease such as vulvovaginal atrophy and other sexual disorders reduce the life quality.
Quality of life: POI negatively affects the quality of life (QoL) and psychological health of afflicted women. High rate of depression and low self-esteem has been reported in these women due to the loss of fertility as well as the consequent sexual disorders. Brain disorders : early estrogen deficiency has deleterious effects on the brain and correlates with nervousness, anxiety, depression, irritability, lack of concentration, insomnia, restlessness, loss of concentration, etc., in POI .
Neurological complications of POI, such as declining short-term memory, cognitive function, and dementia and neurological effects and overall reduced life expectancy. A long-term continuation of the POI would be strongly have effect on., cognitive impairments, urogenital and sexual disorders, infertility, lower quality of life, and twofold age-specific mortality rate.

Inm-8: Managing Premature Ovarian Insufficiency

Mirghavameddin NS

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

Premature ovarian failure (POF) is defined as 4–6 months of amenorrhea in women under the age of 40, who have elevated follicle-stimulating hormone (FSH) and low estradiol levels.
POF is a clinical term used to describe a condition in which women present with amenorrhoea, hypergonadotropic hypogonadism, and infertility under 40 years old, which are mainly characterized by ovarian granulosa cell inflammation and death.
Because most diseases in women occur after menopause, the onset of menopause heralds an important opportunity to institute prevention strategies for prolonging and improving the quality of life for women.
The most common words women use to describe how they feel in the hours after getting the diagnosis of POI are "devastated," "shocked," and "confused."
Practice Nurses and midwives are often the first health professional a woman with premature ovarian insufficiency will see. A practice nurse may be the first encounter a woman has when she presents with amenorrhoea, so understanding her unique concerns and promptly identifying the underlying cause of menstrual changes is essential to improving her long-term physical and psychological health.
Vasomotor symptoms or hot flushes may persist for 10 or more years, with bothersome flushes occurring for about 7 years.
There is a need for an evidenced-based integrated program to assist women with premature ovarian insufficiency (POI) in navigating the transition to acceptance of the diagnosis, ongoing management of the condition, and ongoing maintenance of wellness in the presence of the disorder. A health-centered approach can gradually replace the disease-centered approach and put patients in partnerships with professional health-care providers. Ideally, the journey transitions each patient from seeing herself as a victim, to a survivor, to a woman who is thriving.
Women with POI face a disruption in their pursuit of childbearing, a life goal that is central for many people. Practice

Nurses and midwives can help patients deal with this disruption by helping them build on the inherent positive psychological resources they possess, such as their flexibility in setting goals and their desire to define purpose in their life. Also, many women report that their spirituality or faith is a resource that can assist them in the psychosocial transition induced by the diagnosis of POI.
For women who get a diagnosis of POI, the journey from recovery, to self-management, to wellness presents a series of challenges, hurdles, and frustrations. Wellness can be found by moving from a place of fear and doubt to a place of hope and confidence. For many women the ultimate challenge is finding a place of self-acceptance with regard to their family plans.
Women with spontaneous POI were reported to score adversely on all measures of psychological functioning with higher negative feelings such as "blue mood", despair, anxiety, and depression or had a negative impact on their self-image and confidence. Marital relationship and social support were reported to be significantly lower in POI patients. Social relationships were found to have a negative influence of sexual function such as arousal, orgasm, satisfaction and pain.
POI is a serious and incurable chronic disease. The diagnosis is more than infertility and affects a woman's physical and emotional well-being. Management of the condition must address both.The Midwifery care planning goals and Management for patients with menopause aim to promote symptom management and improve quality of life. This includes providing education and support on managing menopausal symptoms, such as hot flashes, mood changes, and sleep disturbances, while also focusing on preventive measures for long-term health, including bone health and cardiovascular health.

Inm-9: Endocrine Disorders of Premature Ovarian Insufficiency

Hosseini R[1, 2]

1. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran.
2. Department of Basic and Population Based Studies in NCD, Reproductive Epidemiology Research Center, Royan Institute, ACECR, Tehran, Iran
Email: rohosseini@yahoo.com

Premature ovarian insufficiency is a clinical syndrome defined by loss of ovarian activity before the age of 40. POI is characterized by menstrual disturbance (amenorrhea or oligomenorrhea) with raised gonadotropins and low estradiol.The prevalence of POI is approximately 1%. Population characteristics such as ethnicity may affect the prevalence. Autoimmune disorders are more frequent in POI than in the general population, and POI is more frequent in women with certain autoimmune disorders. The most clinically important association is with autoimmune Addison's disease, in the context of autoimmune polyendocrine syndrome (APS).
POI may also be associated with localized or systemic non-adrenal disorders, such as thyroid diseases, hypoparathyroidism, hypophysitis, type 1 diabetes mellitus, and non-endocrine autoimmune diseases. Adrenocortical antibodies (ACA) and more specifically 21-hydroxylase autoantibodies (21OH-Ab) appear to be the marker with the highest diagnostic sensitivity for autoimmune POI. Screening for 21OH-Ab or alternatively (ACA) should be considered in women with POI of unknown cause or if an immune disorder is suspected. Refer POI patients with a positive 21OH-Ab/ACA test to an endocrinologist for testing of adrenal function and to rule out Addison's disease.
POI is associated most commonly with thyroid autoimmunity (14–27%) when adrenal autoimmunity is absent. Screening for thyroid (TPO-Ab) antibodies should be performed in women with POI of unknown cause or if an immune disorder is suspected. In patients with a positive TPO-Ab test, thyroid stimulating hormone (TSH) should be measured every year.
There is insufficient evidence to recommend routinely screening POI women for diabetes.
The effect of POI-associated estrogen deficiency on bone is among the most clearly established adverse consequences of the condition. Women with POI have been shown to have reduced BMD and possibly an increased risk of fracture later in life.
Measurement of BMD at initial diagnosis of POI should be considered for all women, but especially where there are additional risk factors. If BMD is normal and adequate systemic estrogen replacement is commenced, the value of repeated DEXA scan is low. If a diagnosis of osteoporosis is made and estrogen replacement or other therapy initiated, BMD measurement should be repeated within 5 years.

Inm-10: The Effect of Sexual Disorders in ART Success Rate

Narimani N

Department of Urology, Hasheminejad Kidney Center, Iran University of Medical Sciences, Tehran, Iran
Email: nima_dr2001@yahoo.com

About 15% of couples are suffered from infertility and its related burden (psychological, economical, etc.). it has been estimated that in more than 50% of cases, the cause of infertility may be attributed to male factors. Erectile , ejaculatory and orgasmic dysfunctions are common in infertile men. The prevalence of aforementioned dysfunction is about 10 to 15% in these men. Erectile function may be an indicator of male general wellbeing. In this regard, erectile dysfunction and male infertility may be considered as poor health status. There are several systemic disease (e.g. cancerous, auto immune, cardiovascular disease, etc.) which may lead to both sexual dysfunction and infertility. On the other hands medication for treatment of such disease may also lead to erectile dysfunction. The drug and substance abuse such as androgens in sports, body building etc. can lead to infertility (azoospermia) and erectile dysfunction (in withdrawal period) as well. On the other hands the heavy psychological burden of male infertility may lead to erectile dysfunction. Therefore in infertile men precise investigation about general illnesses, medication and psychological status seems to be necessary.

Inm-11: Nursing and Midwifery Ethics and Medical Ethics in Nursing

Omani-Samani R

Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: r.samani@gmail.com

Medical ethics codes are not just for physicians but are for anybody involves in the treatment protocols. Nurses and midwives

are very close to the patient and are the first person to whom the patient always complains. So, patients are psychologically attached to their nurses and midwives and ask their questions from them always.
Many nursing societies like The International Council of Nursing and American Nurses Association have their own codes of ethics. Differences between these types of codes comparing to medical ethics codes are just the position of nurses in treatment procedures.
It seems that the 4 principles of medical ethics and 7 principles of professional ethics are same for nurses and physicians but in application of these principles in the midwives and nurses' job there can be some differences. In Nursing code of ethics basic responsibility of the nurses are abstracted to four items: to promote health, prevent illness, to restore health and decrease suffering and pain. These principles are based on three believes: respect for life, human dignity and human rights. But all of the nursing and midwifery responsibility can be abstracted to one beautiful word: "Care". For this reason, the care ethics was described by Carol Gilligan which is the philosophy of care and nursing and midwifery. For this reason, the image of a nurse and midwife who must emphasize empathy and compassion is normally "a woman", although there are several highly skilled male nurses working in the hospitals.
So, the principles of nursing and midwifery ethics are very similar to medical ethics with adding "care" as the main aim of the nurse and midwife rather than treatment that would be the main aim of physician.
Keywords: Nurse and Midwife Ethics, Nurses and Midwives ethical Codes, Care Ethics, Nursing and Midwives

Inm-12: Polycystic Ovarian Syndrome and Pregnancy

Pooransari P

Shohada Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: parichehrpooransari@gmail.com

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting up to 8-13% of reproductive-aged women. Polycystic ovary syndrome (PCOS) is associated with a higher risk for pregnancy and birth complications according to the specific features associated with PCOS.
The association of PCOS with pregnancy and birth complications varies by PCOS phenotype, target population, ethnic background, self or family history of metabolic, reproductive, and potentially psychological complications during or outside pregnancy, and women's lifestyle. In pregnancy, obesity is an independent risk factor for complications, as is excess gestational weight gain. Women with PCOS have a higher gestational weight gain compared with women without the diagnosis . This also contributes to their increased risk for pregnancy complications. There are no guidelines on antenatal care that specifically target women with PCOS. Most recommendations are indirect and on the basis of the evidence that pregnancy complications such as GDM, hypertension, preeclampsia, and preterm deliveries are more frequent among women with PCOS. Optimally, antenatal care should aim to detect and, if possible, modify risk factors before and during pregnancy.
It is, however, important to keep in mind that most women with PCOS have uneventful pregnancies. Diet and lifestyle modifications to optimize weight-control before pregnancy and adjusting treatment for co-morbidities is of importance. Both preconception and antenatal screening for diabetes and hypertension is recommended. Metformin should not be used routinely in pregnancy. Decreased breastfeeding ability in mothers with PCOS is probably linked to preexisting overweight and obesity.

Inm-13: Subclinical Hypothyroidism and Pregnancy

Ramezani Tehrani F, Nazarpour S

Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Fah.tehrani@gmail.com

During pregnancy, normal thyroid function undergoes significant changes to adapt to the fetus and mother's requirements. Considering these changes and providing a trimester-specific reference range for thyroid hormones is highly required for precise diagnosis of thyroid disturbances in pregnancy.
Subclinical hypothyroidism (SCH) is the second most common endocrine disorder in women of reproductive age; its prevalence is varied from 2-13%, according to the various cut-off values for TSH, age, ethnicities, iodine intake, and lifestyle. Despite the solid evidence for the adverse effects of overt hypothyroidism on pregnancy outcomes, the impact of SCH on pregnancy is questionable. Furthermore, while LT4 treatment is highly suggested for pregnant women with overt thyroid dysfunction, there is no consensus on beneficiary effect of treating SCH women in terms of improvement of pregnancy outcomes. Several studies revealed a higher rate of pregnancy complications in pregnant women with SCH who has thyroid autoimmunity and/or iodine insufficiency. The beneficiary role of LT4 treatment in women with SCH, and the impact of TPOAb or Iodine status on pregnancy outcomes in women with SCH is unclear.

Inm-14: Familiarity with Jurisprudence Aspects of Infertility Clinics

Reza Nia Moaelm R

Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: Moallem1380@gmail.com

Inm-15: A Look at The Psychological Issues of Patients with Premature Ovarian Failure

Sabeti SH

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: sh.sabeti73@gmail.com

Premature ovarian failure unfortunately is not rare these days, young female who confront with POF will know they have lots of medical issues to future, like hormones problem, osteopenia, cardiovascular problem, cognitive disorders, metabolic disorders, sexual dysfunction, and most important; infertility.
This bad news affects them such as main grief as a female they lost their selfesteem and self body image, depression will be occurred then.

For prevention from cognitive and psychological disorders, paitient should receive consultation and adequate information about premature ovarian insufficiency (POI).
There are many methods to support and treatment their psychological issues.
We could have consultation session to understand their psychological problem due to POF, after diagnosis try to approach them with consultation medical treatment.
Group therapy for POI female is so beneficial methods for same women who can share their feelings and thoughts to this silent grief.

Inm-16: The Role of Midwifery Counseling in Improving Sexual Satisfaction in Infertile Patients.

Sadeghi M

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

This essay underscores the relationship between negative emotions, sexual satisfaction, and fertility outcomes. It discusses how negative feelings such as anger and stress can significantly diminish sexual satisfaction among infertile couples, thereby intensify the challenges they face. therefor the psychological impact of these emotions on both partners should be highlighted. Therefore, midwives and doctors play a crucial part in providing emotional support and counseling to mitigate these effects. Through their guidance, these healthcare professionals can help alleviate stress and anger, promote a healthier emotional state that ultimately contributes to improved sexual satisfaction and potentially enhanced fertility prospects for the couples involved.

Inm-17: The Nurse's Legal Liabilities in Fertility Clinics, in Treating and Caring for Patients

Shariati Nasab S

Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
Email: shariatina@ut.ac.ir

In a general classification, nurse's legal liabilities in treating and caring for patients can be divided into three categories: "Tort Liability", "Criminal Punishment" and "Administrative Punishment". Tort liability arise when there is a negligence or fault in treatment, which is not subject to a criminal charge, but causes damage to the patient (whether tangible or intangible); such as mistreatment, or unintentional damages; provided that: 1. The nurse's action be illegal; 2. Nurse's act Leads to patient's damage; and 3. There be a causal relationship between the patient's damage and the nurse's action. Criminal punishment is in cases where the law, explicitly determines punishment for a specific act or omission of an act. Some legal examples of such cases include: leaving a legal duty in treatment or care (Article 295 of the "Islamic Penal Code"); and proceeding to the physician's wrong prescription, knowing its wrong (Note 1, Article 496 of the same Code); and violation of superiors' orders (for example, Article 23 of the "Prevention of Sexually Transmitted Diseases and Infectious Diseases Act"). Finally, a disciplinary violation is related to infraction from jurisprudential and legal standards, and breaching guild and professional regulations, or negligence in performing duties, which, if committed, will result in disciplinary punishments; such as reprimand or deprivation of profession (Article 28 of the "Nursing Organization of Islamic Republic of Iran Act", and Articles 23 and 24 of "Medical Council of Islamic Republic of Iran Act"). Generally, the tort and criminal liability cases, at the same time, may lead to disciplinary punishments.

Inm-18: Sexual Disorders in Infertile Women

Shiva M

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

Infertility affects different areas of the couple's life. Unsuccessful family planning has a negative impact on individual's feelings, and many couples describe the period of diagnosis and treatment of infertility as the most stressful period of their life. The ability of reproduction is closely connected with self-image, self-respect, and sexuality. Sexual intercourse may lose its spontaneity and erotic value because the main aim becomes conception. This may affect the ability for intimate sexuality and can provoke certain sexual dysfunctions. Sexual dysfunctions can be a reason for infertility or can be triggered by infertility.
Identifying sexual disorders as relevant considerations in the context of infertility and exploring their impact during the entire course of diagnosis and treatment constitute an important contribution to comprehensively care for the couples concerned. Counselling should focus on preventing the onset and aggravation of sexual disorders. As sexuality represents a major component of quality of life and of partnership, such support may improve not only the current overall wellbeing but also th chances of a satisfactory long-term partnership and family life.
The majority of studies confirmed the negative effects of infertility on couples' sexuality. This impact was found to be most pronounced in women with secondary infertility. The results also imply that infertility treatment may increase the risk of sexual dysfunctions. This gives certain indices for clinical improvements of infertility management. Couples who go through the process of infertility treatment are never evaluated for sexual dysfunctions11, but they should be, and they should also be offered help in case of diagnosis of sexual dysfunction. In the case of unwilling childlessness, sexual therapy should be offered to clients, so that the quality of sexuality is at least the same as before the infertility treatment.

Oral Speaker

Onm-1: Recurrent Implantation Failure and Sexual Function in Infertile Iranian Women: A Comparative Cross-Sectional Study

Ghorbani S[1*], Abedi P[2], Hekmat Kh[2], Ghanbari S[3], Dibavand N[4]

1. Department of Midwifery, Jundishapur University of Medical Sciences, Ahwaz, Iran
2. Department of Midwifery, Reproductive Health Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran
3. Department of Biostatistics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran
4. Fertility Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran
Email: Hekmat1346@gmail.com

Background: Recurrent implantation failure (RIF) which means failing to implant after two or more high-quality embryo transfer cycles, affects 3 to 5% of women worldwide. The aim of this study was to assess the relationship between recurrent implantation failure and sexual function in infertile Iranian women.
Materials and Methods: This was a comparative cross-sectional study on 180 infertile Iranian women (90 infertile women with recurrent implantation failure and 90 infertile women who did not start infertility treatment). A demographic questionnaire and the Female Sexual Function Index were used for data collection. Data were analyzed using Chi-square, independent t-test, and multiple linear regression.
Results: The mean scores of different domains of sexual function (desire, lubrication, arousal, orgasm, pain, and satisfaction) were significantly lower in the group with RIF compared to the group without RIF. The total score of sexual function was significantly lower in the RIF group compared with the group without RIF (23.11 ± 2.24, vs. 25.99 ± 2.35, P<0.001).
Conclusion: The results of this study showed that women with RIF had significantly lower sexual function than that in women without RIF. Therefore, sexual function issues should be treated as an important component of comprehensive care. This study did not measure the impact of economic factors on sexual function, however, the majority of the sample were classified as having weak or moderate economic status and this, along with the high cost of infertility treatments, could potentially have played a role in the participants' experience.
Keywords: Infertility, Recurrent Implantation Failure, Sexual Function,

Onm-2: The Role of Men's Forgiveness in Marital Satisfaction and Coping Strategies of Infertile Iranian Women

Hasanpoorazghady SB[1*], Safari S[2]

1. Department of Midwifery and Reproductive, Nursing and Midwifery Care Research Center, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran
2. Department of Midwifery and Reproductive, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran
Email: hasanpoorbatool@yahoo.com

Background: Infertility and its related problems create tensions in infertile women, which may lead to reduced marital satisfaction and the use of ineffective coping strategies. Considering the important role of forgiveness, marital satisfaction and effective coping strategies in the quality of life of infertile couples, and taking into account the growing number of Iranian infertile couples, this study was conducted to determine the relationship between men's forgiveness, marital satisfaction, and coping strategies of infertile Iranian women.
Materials and Methods: This cross-sectional study included 200 Iranian infertile couples. The research environment was the most equipped infertility center in the west of Iran. Sampling was continuous. Data collection tools used included a self-generated demographic and fertility questionnaire, the Family Forgiveness Scale (FFS), the Index of Marital Satisfaction (IMS), and the Ways of Coping Questionnaire-revised (WOCQ-R).
Results: Husbands' forgiveness had a significant direct relationship with the marital satisfaction of infertile women (r=-0.27, P<0.001). However, there was no significant correlation between Husbands' forgiveness, emotion-focused, and problem-focused coping of infertile women. Among the subscales of forgiveness, only the subscale of recognition had inversely correlated with the emotional coping of infertile women.
Conclusion: The results showed that the higher the forgiveness of husbands, the higher the marital satisfaction of infertile women. Also, with the increase of husbands' forgiveness in the recognition subscale, the use of emotion-focused coping decreased in infertile women. Based on the results of empowering the husbands of infertile women with forgiveness skills, it is possible to take a step towards marital satisfaction and thus improve the quality of life of infertile women.
Keywords: Coping Strategies, Forgiveness, Infertility, Marital Relationship

Onm-3: Sexual Function and Satisfaction Among Women Undergoing *In Vitro* Fertilization (IVF) in The West of Iran: A Cross-Sectional Study

Jenabi E

Department of Midwifery, Hamadan University of Medical Sciences, Hamadan, Iran
Email: en.jenabi@yahoo.com

Background: Considering that no study has been conducted regarding sexual function and satisfaction in infertile women who are undergoing treatment. The present study was conducted to investigate sexual performance and satisfaction among women undergoing *in vitro* fertilization (IVF) in Western Iran.
Materials and Methods: This cross-sectional study was conducted on 170 women from December 2022 to March 2023. The participants were selected by the simple random sampling method. The participants were included in the study as available sampling. Women completed the demographic questionnaire, FSFI (female sexual function index), and Linda Berg's Sexual Satisfaction Questionnaire. For data analysis, we applied the Stata version 14 (StataCorp, College Station, TX) and we considered the P value less than 0.05 significant.
Results: There was a significant relation between husband occupations, economic status, marriage duration, period of infertility, cause of infertility, and intercourse times with sexual satisfaction (P<0.05). The highest percentage of the obtained score was related to pain (51.6%) and the lowest was related to desire (38%). All of the patients with weak sexual function had weak sexual satisfaction and 60.19% of the patients with good sexual function had good sexual satisfaction (P<0.001).
Conclusion: Women with weak sexual function had weak sexual satisfaction. Policymakers should consider strategies such as counseling and psychological support for women during the treatment process to help them cope with their problems, especially psychological problems.
Keywords: Sexual Function, *In Vitro* Fertilization, Cross-Sectional Study, Iran, Satisfaction

Poster Presentation

Pnm-1: Which Intervention Is More Effective in Assisted Reproductive Technology Success: Relaxation or Journaling?

Amini L*, Najd Gholami Z

Department of Midwifery, Nursing Care Research Center (NCRC), Tehran, Iran
Email: amini.l@iums.ac.ir

Background: Todays, many treatment strategies are considered to increase the success of ART. The present study aimed to comparing the effect of relaxation and journaling on the treatment success of ART methods.
Materials and Methods: This randomized clinical trial study with a control group was done on 90 infertile women with age 20 to 40 years referred for treatment to the Shahid Akbarabadi infertility clinic. Sampling was done continuously and the allocation of samples in studies groups was done randomly. In the relaxation group, relaxation exercises were performed once a day for 30 minutes consecutively until the day of puncture. For journaling, the participants began to write, as soon as they woke up. The intervention continued until the day of egg retrieval.
Results: The findings of this study showed that the most women in all groups were 30-39 years old and the studied groups had no statistically significant differences in terms of demographic characteristics. The results did not show any significant differences between two groups in terms of retrieved follicles (7.40 ± 7.22 vs. 8.07 ± 6.66 in journaling and relaxation groups respectively) and the mean number of embryos (3.77 ± 2.83 vs. 4.07 ± 4.05 in journaling and relaxation groups respectively) groups.
Conclusion: Although the results of this study didn't show any significant differences between two groups in term of studied variables, but since previous studies showed effectiveness of relaxation, it can be concluded that journaling is effective too.
Keywords: Infertility, Journaling, Relaxation, Writing

Pnm-2: The Relationship between Sexual and Marital Satisfaction Level and The Causes of Infertility in Infertile Couples

Arabipoor A[1*], Mansouri M[2], Heydar Z[3], Lotfi R[4], Rahimzadeh Kiwi M[5], Tahamtan T[6]

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Shahid Rast Ravesh Comprehensive Health Services Center, Alborz University of Medical Sciences and Health Services, Karaj, Iran
3. Department of Clinical Research Development, Mahdieh Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Midwifery and Reproductive Health Department, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
5. Social Determinants of Health Research Center, Public Health School, Alborz University of Medical Sciences, Karaj, Iran
6. Department of Medical Education, Iran university of Medical Sciences, Tehran, Iran
Email: arezoo.arabipoor@gmail.com

Background: To investigate the effect of infertility on sexual and marital satisfaction of infertile couples. The present study designed to increase the knowledge of infertile couple regarding psychological and sociocultural problems of fertility and improve the health policy plans to increase the sexual and marital satisfaction of infertile couples.
Materials and Methods: This cross-sectional study was carried out at Rooyesh infertility center of Alborz Province from March 2014 to March 2015. All infertile couples with at least primary education were enrolled. Data collection instrument was a questionnaire which was given to each partner separately by the researcher. The first part of data collection form included demographic questionnaire (13 questions) and the second part was the marital satisfaction (18 questions) and the third part was the assessment of sexual satisfaction (11 questions). The scores of couples' marital and sexual satisfaction were compared among couples with different cause of infertility by using appropriate statistical tests.
Results: Overall, 174 couples were evaluated during the study period. The significant correlation was found between couples 'age and education and income (P=0.02 = P. 01, P<0.0001, P and P =0.04, respectively). There is no association between demographic characteristics and couples' sexual satisfaction. In this study, the cause of infertility in 27% of couples were diagnosed as male factor (n=47), 30.5% female factor (n=53), 20 both male and female factors (n=35) and %22.5 unexplained infertility (n=39). No significant relationship was found between causes of infertility and couples' marital and sexual satisfaction.
Conclusion: On the basis of present results, it seems that the cause of infertility has no impact on couples' marital and sexual satisfaction. However, to confirm these results, more studies with larger sample sizes are required.
Keywords: Cause of Infertility, Marital Satisfaction, Sexual Satisfaction

Pnm-3: The Effects of Life Style on The Success Rate of *In Vitro* Fertilization

Ghorbani Kolahi B

Department of Midwifery, Islamic Azad University of Tehran, Tehran, Iran
Email: Baharghorbanik@gmail.com

Background: *In vitro* fertilization (IVF) is an associated reproductive technology (ART) which is the main ART used to treat infertility. One option for improving IVF success is to develop a healthy life style. Diet, physical activity and mental health could be altered to enhance the chance of IVF success. Smoking, alcohol consumption and bad nutrition can reduce the IVF success. The aim of this evaluation was to estimate the impact of life style on the IVF outcomes.
Materials and Methods: This review has been conducted based on analysis of available literature indexed in PubMed database between 2013 and 2023. Specific keywords including

"*In vitro* fertilization", "Associated reproductive technology", "Life style", "Success of ART" and Physical activity" have been used.
Results: Studies suggests that there is an important impact of life style factors on IVF outcomes. Cigarette can affect reproductive outcomes including decreasing sperm parameters and ovarian reserve as reflected by low antral follicle count (AFC) and anti mullerian hormone (AMH) levels. Some reviews suggest that sperm parameters were reduced in alcohol consumers due to DNA damage. The adherence to the healthy diet has higher likelihood of live birth. Physical activity can improve insulin sensitivity and mental health that have beneficial effects on fertility.
Conclusion: In summary, there is an important effect of life style factors in IVF outcomes. Diet, physical activity, stress and smoking were identified as the most modifiable factors. Appropriate counseling could result in higher success rate of ART. It is necessary to mention that we need more studies to correlate the impact of life style on IVF outcomes.
Keywords: ART, IVF, Life Style, Physical Activity, Success of ART

Pnm-4: Relationship between Religious Coping and Health-Promoting Lifestyle Among Iranian Infertile Women

Hasanpoorazghady Seyedeh B[1*], Gheysari A[2], Farahaninia M[2]

1. Department of Midwifery and Reproductive, Nursing and Midwifery Care Research Center, School of Nursing and Midwifery., Iran University of Medical Sciences, Tehran, Iran
2. Department of Community Health Nursing, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran
Email: hasanpoorbatool@yahoo.com

Background: Infertility is recognized worldwide and across cultures as a stressful, critical and threatening experience of personal, marital, family, and social stability. The role of religion as a source of support in facing health-related problems has been confirmed in some studies. This study aimed to determine the relationship between religious coping and health-promoting lifestyle (HPL) in Iranian infertile women.
Materials and Methods: A cross-sectional correlational study was performed on 177 infertile women referring to Sarem super specialized infertility treatment and research center in Tehran. Sampling was done continuously. Data were collected using demographic information form, the Iranian Religious Coping Scale (IRCS), and Health-Promoting Lifestyle profile-II (HPLP-II).
Results: The findings showed the highest mean score was active religious coping (7.86 ± 2.39), and the lowest mean score was passive religious coping (2.89 ± 1.76) followed by negative feelings toward God (3.95 ± 2.07). The mean score for health-promoting lifestyle was (128.34 ± 13.46; the score range was 52-208) lower than the median score of the scale. There was a significant inverse relation but weak between negative feelings toward God and health-promoting lifestyle (r =-0.19; P =0.013) and its three subscales namely physical activity (r=-0.18; P=0.019), nutrition (r=-0.21; P=0.011), and interpersonal relationships (r=-0.21; P=0.01) as well as passive religious coping and interpersonal relations (r=-0.18; P=0.029).
Conclusion: Considering that the mean HPL score of infertile women was lower than the median score of the scale, community-oriented education and care programs should be considered to improve HPL in infertile women. It is also suggested to teach and strengthen positive religious coping strategies to improve HPL.
Keywords: Religious Coping, Health-Promoting lifestyle, Infertile Women

Pnm-5: Sexual Dysfunction in Women with Endometriosis: A Narrative Review

Heidari Sarvestani M[*], Noroozi M

Department of Midwifery and Reproductive Health, School of Nursing and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran
Email: noroozi@nm.mui.ac.ir

Background: Endometriosis is a chronic disease with a prevalence of 6-10% in women of reproductive age. The negative impact of endometriosis on intimate relationships and sexual function has been reported in a significant percentage of patients. This study was conducted with the aim of identifying sexual dysfunction in women with endometriosis based on a review of the studies.
Materials and Methods: This narrative review searched all English studies indexed in databases such as PubMed, Google Scholar, Scopus and ISI Web of Science from 2017 until now with the keywords "sexual dysfunction, endometriosis and sexual health". After reviewing the studies, among the 60 studies, finally 25 studies were selected for the final evaluation.
Results: Based on the studies, sexual health is one of the neglected areas in the medical care of women with endometriosis. The prevalence of sexual dysfunction in these women is 30-70% and is almost double as compared to those with other gynecological disorders. About two-thirds of affected women have various sexual dysfunction such as hypoactive sexual desire, decreased lubrication, dyspareunia, orgasmic disorders, and decreased sexual satisfaction, which lead to decreased quality of life, increased psychological disorders, and interpersonal and marital problems.
Conclusion: Considering the various problems related to sexual function in women with endometriosis, it is necessary to focus the treatment not only on the symptoms of the disease, but also in relation to the identification of sexual dysfunction and its treatment in these women.
Keywords: Endometriosis, Sexual Dysfunction, Sexual Health, Women

Pnm-6: Iranian Gamete Donors' Experiences of Quality of Care in Donation Process: A Qualitative Study

Iranifard E[1*], Ebrahimzadeh Zagami S[2, 3], Amirian M[4], Latifnejad Roudsari R[2, 3]

1. Student Research Committee, Mashhad University of Medical

Sciences, Mashhad, Iran
2. Nursing and Midwifery Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Obstetrics and Gynecology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email:rlatifnejad@yahoo.com

Background: The services provided to gamete donors by fertility clinics should not be limited to counseling. Gamete donors go through a complex process of decision-making for donation, which will have long-term effects on their personal and social life. In order to provide them with the services and care they need; it is important to understand the experience of gamete donors in relation to the care provided to them. The aim of this study was to explore the quality of care experienced by Iranian gamete donors through the donation process.
Materials and Methods: A descriptive qualitative study was conducted in three fertility centers, each located in a different city in the central and eastern parts of Iran. Participants including one embryo donor, two known egg donors, two commercial egg donors, one commercial sperm donor, and a care provider; entered the study through purposeful sampling within six months. Data were collected via semi-structured interviews, and conventional content analysis based on Graneheim and Lundman approach was used for data analysis.
Results: The main category of "quality of care in donation process" emerged. "Presence of an inefficient structure", "facing difficulties through donation process", and "satisfaction with the provided care" were three subcategories for the main category.
Conclusion: The current structure of the donation process in Iran is suboptimal. It seems that a comprehensive care program and/or guideline at the national level is needed to improve the quality of care provided to donors in order to ensure their satisfaction.
Keywords: Embryo Donation, Oocyte Donation, Quality of Care, Third-Party Reproduction, Sperm Donation

Pnm-7: Domestic Violence Among Infertile Women in The West of Iran: A Cross-Sectional Study

Jenabi E*, Abdoli S, khazaei S

Department of Midwifery, Hamadan University of Medical Sciences, Hamadan, Iran
Email:en.jenabi@yahoo.com

Background: Due to the importance of domestic violence and on the other hand, no study has been conducted on domestic violence among barren women in western Iran. Therefore, the purpose of this study is to investigate domestic violence among barren women in western Iran.
Materials and Methods: This research was a cross-sectional study that was conducted at the infertility clinic in Hamadan. In total, 200 eligible infertile women were included in the study. Domestic Violence Questionnaire (DVQ) was completed for all samples. Stata statistical Software (version 17) was used for data analysis. $P \leq 0.05$ was considered statistically significant.
Results: In this study, 200 infertile women with an average age of 31.48 ± 4.57 years were examined. The average duration of the infertility period in them was 1.9 ± 0.76 years. In terms of education, about 66% of them and 69% of their spouses had high school or diploma education. In 72.5% of cases, the cause of infertility was female. 59.4% of women had experienced domestic violence. The highest percentage score of violence was related to the emotional area (67.5%) and the lowest percentage score was related to the sexual area (43.63%). There was a statistically significant relationship between the age, education of the spouse, and the number of sexual intercourses with the level of violence in women.
Conclusion: In the present study, 59.4% of women were exposed to violence in various forms. The association between domestic violence with age, spouse's education, and the number of intercourses was statistically significant.
Keywords: Cross-Sectional Study, Domestic Violence, Infertility, Iran

Pnm-8: The Role of Counseling in The Treatment of Infertile Couples

Kalhor M*

Department of Midwifery, School of Nursing and Midwifery, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email:mkalhor20@gmail.com

Background: Infertility with damage to various dimensions of health leads to tension in the individual and interpersonal relationships of couples. It seems that counseling can play an important role in improving the treatment process of infertile couples. The present study aims to investigate the role of counseling in infertile couples.
Materials and Methods: The study reviewed several significant articles in the field between 2010 and 2023. Reviews in the journal and PubMed, Science Direct, Elsevier, ProQuest, Willy, springer, and google scholar databases with keywords Infertility, counseling interventions, couples, and group counseling. The results were analyzed and ranked in a schematic way. For statistical analysis, statistical tests were used.
Results: The results of the present study showed that several main factors play a role in this consultation. The results show that the different methods of counseling (Group counseling, behavioral therapy, group psychotherapy) and the effect of the intervention should be individual and according to the special conditions of each infertile couple. Also, the type of intervention, the number of sessions, and the content of counseling programs should be according to the individual, socio-economic, and cultural conditions of each one. Prepared and presented by couples.
Conclusion: Counseling methods and practical techniques can be helpful in changing thoughts, attitudes, and beliefs. Therefore, the use of counseling methods can play a significant role in the health of infertile couples.
Keywords: Infertility, Counseling Interventions, Couples, Group Counseling

Pnm-9: Sperm DNA Fragmentation and Recurrent Pregnancy Loss

Kouhestani S

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

Background: Recurrent pregnancy loss (RPL) is a common, yet intensely traumatic event. Although the exact definition of RPL remains a topic ongoing debate, it is commonly defined as either =>2 failed clinical pregnancies as documented by ultrasonographic or histopathologic examination. Research has primarily focused on maternal causes for RPL, but there is growing evidence demonstrating the impact of male factors. Through advances in molecular genetics, sperm DNA integrity has been shown to affect fertilization, subsequent embryo development, implantation, and pregnancy. The purpose of this study was to determine whether there is a significant relationship between sperm DNA fragmentation(SDF) and recurrent pregnancy loss.
Materials and Methods: In this review study, the PubMed and google scholar databases were searched, with the keywords recurrent pregnancy loss, recurrent miscarriage, sperm DNA fragmentation. The articles from 2000 to 2023 with were included in the study. After reviewing the studies, among the 34 studies, finally 17 studies were selected for the final evaluation.
Results: According to the results obtained from the studies, SDF is one of the important causes of RPL, and couples with a history of RPL showed a higher prevalence of SDF and poor progressive sperm motility.
Conclusion: These findings suggest that the DNA fragmentation test has a certain predictive value in assessing the possible risk of RPL. However, given the significant heterogeneity between studies and the lack of prospective pregnancy outcome data, more large prospective studies are needed.
Keywords: Recurrent Pregnancy Loss, Recurrent Miscarriage, Sperm DNA Fragmentation

Pnm-10: The Effect of ICSI on Male Fertility: A Review Study

Mokhtari F[1, 2*], Rezaei A[3]

1. Nursing and Midwifery Care Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
2. Nursing and Midwifery Care Research Center, Faculty of Nursing and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Radiology, Isfahan University of Medical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
Email: Mid.mokhtari@yahoo.com

Background: The use of Assisted Reproductive Technology, mainly includes *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), has increased. There are still concerns about the use of ICSI on the fertility of men born from this method. Therefore, the aim of this study was to investigate the effect of ICSI on male fertility.
Materials and Methods: In this review, in order to find related articles, the Web of Science, Scopus, ProQuest, PubMed databases, and the Persian Irandoc, SID, Magira databases, and the Google Scholar search engine to find studies published in the period of 1970-2023 using English keywords: Assisted Reproductive Technology, Intracytoplasmic sperm injection, infertility, fertility and male and their Persian equivalents were searched.Among1317 articles,14 articles were eligible for research and were selected for this study.
Results: ICSI boys are at risk of inheriting testicular dysfunction from their fathers. ICSI boys are exposed to puberty problems, especially late puberty. There is a relationship between the later onset of puberty and impaired reproductive health in adulthood, such as reduced semen quality and longer time to pregnancy. Compared to other children, ICSI children are at increased risk of genital abnormalities, urogenital surgery, undescended testicles, and hypospadias. and cryptorchidism, so they can be at risk of impaired spermatogenesis in the later stages of life. The mean concentration, total sperm count, and total motile sperm count were lower in ICSI men than in their peers. Despite these results, most studies have shown that the reproductive capacity of ICSI male children is normal.
Conclusion: Fertility follow-up of children resulting from ICSI shows reassuring results, but it is difficult to distinguish the effects of ICSI from the effects of infertility. Males resulting from ICSI do not seem to show a higher prevalence of fertility disorders than children of infertile couples who conceived naturally.
Keywords: Fertility, Infertility, Intracytoplasmic Sperm Injection, Male

Pnm-11: Reproductive Health of Women with Endometriosis: An Improving Educational Intervention based on The Planned Behavior Theory

Namazi M*, Behboodi Moghadam Z

Midwifery and Reproductive Health, Tehran University of Medical Sciences, Tehran, Iran
Email: masnamazi@yahoo.com

Background: Endometriosis is a chronic deliberating disease with devastating effects on reproductive health. The present study aimed to investigate the impact of education based on the theory of planned behavior (TPB) on the reproductive health of women with endometriosis.
Materials and Methods: This research was a randomized controlled trial performed on 71 women with endometriosis (35 intervention and 36 control groups) referred to the infertility clinic of Imam Khomeini Hospital in Tehran, Iran. The educational intervention based on the structures of the TPB was performed in the intervention group in 4 sessions, weekly for 90–120 minutes. The demographic questionnaire, model constructs questionnaire, and endometriosis reproductive health questionnaire (ERHQ) in both groups were completed in 3 stages (before intervention, 4, and 8 weeks after the intervention). Data were analyzed using SPSS software version 24.
Results: After the educational intervention, TPB values and overall reproductive health of women with endometriosis improved significantly in the intervention group (P<0.05), while

changes were not significant in the control group.
Conclusion: The study results showed that education based on the TPB had positive effects on the reproductive health of patients.
Keywords: Endometriosis, Planned Behavior Theory Reproductive Health

Pnm-12: Investigating The Relationship between Follicular Pattern in Ultrasound and Degree of Menstrual Disturbance

Pourkhani Z*, Jahanian Sadatmahalleh S

Reproductive Health and Midwifery, Tarbiat Modares University, Tehran, Iran
Email: z.pourkhani@modares.ac.ir

Background: Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder that is characterized by the presence of enlarged ovaries with an abundance of small antral follicles distributed about a bright echo dense stroma. the most recent consensus criteria for PCOS have included polycystic ovary syndrome morphology (PCOM) as a relevant marker. Therefore, this study was done with the aim of investigating the relationship between PCOM and menstrual disorders.
Materials and Methods: A literature search was conduct through PubMed, google scholar, ProQuest, Scopus, Springer and Science Direct to identify the relationship between follicular pattern in ultrasound and menstrual disorders.
Results: There are limited data on which features of PCOM inform severity or menstrual disturbance in PCOS. The available studies have reported associations among sonographic markers and menstrual disturbance. Menstrual irregularity may be related to the magnitude of insulin resistance or certain types of serum steroids or to other factors associated with obesity.
Conclusion: There is a relationship between menstrual irregularity and ovarian morphology. It remains unknown if morphology, testosterone or LH causes the menstrual disturbance or if they are co-initiated by an intervening factor.
Keywords: Menstrual Disturbance, Ovarian Follicle, Polycystic Ovary Syndrome, Ultrasound

Pnm-13: Investigating The Relationship between Follicular Pattern in Ultrasound and Metabolic Disturbance in Patients with Polycystic Ovary Syndrome

Pourkhani Z*, Jahanian Sadatmahalleh S

Department of Reproductive Health and Midwifery, Tarbiat Modares University, Tehran, Iran
Email: z.pourkhani@modares.ac.ir

Background: One of the most common disorders for women at childbearing age is polycystic ovary syndrome (PCOS), which affects not only hormones that regulate the normal development of eggs in the ovaries but also other metabolic pathways. While the relevance of polycystic ovarian morphology (PCOM) to the diagnosis of PCOS has been debated. We want to investigate the relationship between ovarian ultrasound patterns and metabolic syndrome in women with PCOS.
Materials and Methods: We performed a review using PubMed-search for peer-reviewed articles related to polycystic ovary syndrome and metabolic syndrome.
Results: Our data support the conclusion that aspects of ovarian morphology do in fact reflect the degree of metabolic disturbance in PCOS. Associations among different sized follicles were consistent with recruit able sized follicles, which reflects the severity of metabolic dysfunction in PCOS.
Conclusion: This study reaffirms the importance of aspects of ovarian morphology to the condition of PCOS. Sonographic markers correlate with key symptomatology in this condition.
Keywords: Metabolic Disturbance, Polycystic Ovary, Polycystic Ovary Syndrome, Ultrasound

Pnm-14: Investigating The Relationship between Follicular Pattern in Ultrasound and Insulin Resistance

Pourkhani Z*, Jahanian Sadatmahalleh S

Department of Reproductive Health and Midwifery, Tarbiat Modares University, Tehran, Iran
Email: z.pourkhani@modares.ac.ir

Background: Polycystic ovary syndrome (PCOS), the most common endocrinopathy in women of reproductive age. Insulin resistance is found in a high percentage of women affected by PCOS. The relevance of polycystic ovarian morphology (PCOM) to the diagnosis of PCOS has been debated. Insulin resistance plays an important pathogenetic role in PCOS. Our aim is to investigate this relationship.
Materials and Methods: A literature search was conduct through PubMed, google scholar, ProQuest, Scopus, Springer and Science Direct to identify the relationship between follicular pattern in ultrasound and insulin resistance.
Results: According to studies, there is identified an association between metabolic parameters and ultrasound pattern in patients affected by PCOS. Ovarian morphology changes dramatically should the primum movens of PCOS development be insulin resistance. Normally, insulin, by way of the classic mechanism of "spill-over," binds insulin growth factor-1 (IGF-1) receptor thereby exerting mitogenic effects on the granulosa and theca. IGF-2 plays a pivotal role in the FSH-mediated proliferation of the granulosa and is hence important for the growth and development of follicles.
Conclusion: These results suggest that insulin resistance could be associated with a specific ultrasound pattern in PCOS patients.
Keywords: Insulin Resistance, Polycystic Ovary Syndrome, Polycystic Ovary, Ultrasound

Pnm-15: The Supportive Needs of Iranian Women with Systemic Lupus Erythematosus: A Qualitative Study

Rezaei E

Department of Midwifery, Faculty of Nursing Midwifery, Tabriz University of Medical Sciences, Tabriz, Iran
Email: rezai520@yahoo.com

Background: The effect of lupus erythematosus (SLE) on

women's health has been ignored in the quality of life of them. This study was conducted with the aim of exploring the expectations and perception of women with SLE regarding for supportive needs.

Materials and Methods: This qualitative research was conducted using 27 semi-structured deep interviews with 19 married women suffering from SLE (15-49 years old) selected through purposive sampling in the referral Rheumatology Center in Iran. Data analysis was performed with a content analysis approach using the conventional method proposed by the Zhang and Wildemuth (2016) by 10 MAXQDA.

Results: The women's perceptions about need for support were categorized in three subcategories included; 1. support from the spouse (most participants stated that women with lupus could cope with the disease challenges if they had a sympathetic spouse and received the required emotional support from their spouses as the only support); 2. support from the family and acquaintances (They can cope with the disease challenges more easily if they have the necessary family support, support and understanding from their husband's family, positive influence of friends and acquaintances, and by setting the friends' spirit), and 3. lack of occupational and social support (They can tolerate their disease by having occupational and social support to escape the thoughts of the disease by working and undertaking different activities, and having financial support to afford the costs of the disease).

Conclusion: It is hoped this research can take a step to enhance the awareness of care providers about these women supportive requires.

Keywords: Systemic Lupus Erythematosus, Women, Qualitative Study

Pnm-16: Ethical Considerations of Sex Selection

Shahbazzadegan S

Department of Midwifery, School of Nursing and Midwifery, Ardabil University of Medical Sciences, Ardebil, Iran
Email: samirashahbazzadegan2000@yahoo.com

Background: Male gender preference is as a cultural characteristic in Iran, especially in some provinces. The health system provides conditions to parents with the means to achieve this. This study was conducted with the aim of surveying ethical considerations of sex selection.

Materials and Methods: The study was performed by referring to the birth statistics of the Iranian Statistics Center and checking the sex ratio during 2016-2022. The obtained statistics were compared with reference books and reliable scientific databases.

Results: Sex selection is a complex subject and many factors such as cultural, biological, social, and ethical factors affect it. Sex selection isn't only an isolated act from reproduction but it is interfering with nature. Today appropriate technology and artificial interferences are available for couples to child's sex selection according to their desires and wishes; unaware of their many consequences. One of them is disturbance of sex balance and ratio. Although the sex ratio is variable in different populations and cannot be fixed at birth, but severe changes in this case are not appropriate.

Conclusion: The continuation of this process causes the violation of the rights of the female gender and leads to negative social consequences such as marriage problems, prostitution, violence against women, and regeneration problems.

Keywords: Ethics, Female gender, Regeneration, Rights, Sex selection

Pnm-17: Impotence Is A Common Problem

Shahbazzadegan S

Department of Midwifery, School of Nursing and Midwifery, Ardabil University of Medical Sciences, Ardebil, Iran
Email: samirashahbazzadegan2000@yahoo.com

Background: Impotence, which is the inability to achieve a full erection or the inability to maintain an erection until the end of sexual intercourse, is one of the main and effective factors for unsatisfying, sexual instinct of couples, and infertility. This study was conducted with the aim of investigating male impotence and infertility.

Materials and Methods: In this study, data was collected with the keywords of sexual impotence, infertility, prevention, and solution, and similar words in Scopus, and PubMed search engines.

Results: Many factors affect male impotence, such as psychological and physical factors and smoking. According to the studies, there is a close connection between cardiovascular diseases, spinal cord injuries and impotence. Also, some surgeries in the pelvic area or around the spine affect the sexual ability with the function of the penis. Penile vessels are an important factor in regulating blood flow and erection. Their health is very important in a person's sexual ability. In the same way, high blood sugar and fat and high blood pressure affect the coronary arteries of the heart and cause a heart attack, then affect the blood vessels of the penis and reduce the ability to get an erection. Stress, use of some drugs including some antihypertensive drugs and some prostate drugs, are effective in impotence.

Conclusion: Impotence is considered a stigma and men need proper counseling and training in this subject. It is necessary for health system policymakers to examine, plan and make effective interventions in the field of sexual health in a general way. Attention should be paid to the existence of centers providing services and care for sexual and reproductive health because mental and physical problems causing impotence can be treated with appropriate techniques.

Keywords: Impotence, Infertility, Sexual Disorders

Pnm-18: The Effect of Weight Loss on Body Image and Binge Eating in Obese Women

Shishehgar F[1*], Vahidi S[2]

1. Department of Midwifery, Faculty of Nursing and Midwifery, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. School of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: shishehgarf@yahoo.com

Background: The majority of previous dietary interventions have more focused on weight loss and the association between weight loss and body image dissatisfaction (BID), and binge eating is less examined. Although some interventions with weight loss noted improvements in BID, some trials found no association between weight loss and reduction in BID. Previous studies that examined the association between weight loss and BID were either cross-sectional studies or bariatric surgery, and few dietary interventions with weight loss have evaluated BID and binge eating disorder (BED) as the primary outcome. This study aimed to determine the relationship between weight loss and BID and binge eating disorder (BED) during 24 weeks of energy-restricted low glycemic index (LGI) diet intervention.
Materials and Methods: 150 overweight and obese women (age= 28.3 ± 4.5 years) participated in 24 weeks of an energy-restricted LGI diet to cause 0.5 kg weight loss per week. The Body Shape Questionnaire (BSQ) was used to assess BID. It is a 34-item self-report questionnaire giving a range from 34 to 204 for the sum of all item scores. Higher scores indicate greater BID. Binge Eating Scale (BES) was used to assess binge eating disorder. It is a 16-item questionnaire, with a final score varying from 0 to 46. Measurements of (BID), (BED), and anthropometric variables were taken at baseline and after 24 weeks. Participants were categorized according to the percentage of weight loss observed during the intervention: 0-4.9% loss (group A), 5-9.99% loss (group B).
Results: After the intervention, there was a significant decrease in body mass index (BMI) (31.7 ±3.3 vs. 28.9 ± 2.8 kg/m^2), and 34% of the sample (n=51) lost: 0%– 4.9% of their baseline weight. There were no statistically significant differences between the two groups on any of the baseline measures. At week 24, both groups had significant improvements in BSQ and BES. Group B had significantly greater reductions in BSQ than Group A (-21.8 vs. -9.2%) (P<0.001). At week 24 of the intervention, Group B had significantly greater improvement in changes in BES (-37.2 vs. -20%) (P<0.001). Percent change in weight loss was significantly correlated with changes in BID and BED.
Conclusion: In overweight or obese women, greater weight loss was associated with greater improvement in BID and BED.
Keywords: Binge Eating, Body Image, Obesity, Weight Loss

Pnm-19: Midwifery Continuity of Care in Infertile Couples: A Systematic Review

Taheri M*, Azizi F

Department of Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
Email: tahery_mi@yahoo.com

Background: Infertility is a distressing condition affecting numerous couples worldwide. The provision of effective care and support is crucial for these couples throughout their journey. Midwifery continuity of care has emerged as a promising model to address the complex needs of infertile couples. This systematic review aims to evaluate the impact of midwifery continuity of care on various outcomes related to the infertility experience.
Materials and Methods: The review utilized a comprehensive search strategy across multiple electronic databases, including PubMed, Embase, and Cochrane Library. Studies published between 2010 and 2022 were included, focusing on the role of midwifery continuity of care in the context of infertility. Relevant articles were screened, and data extraction was performed independently by two reviewers. The selected studies encompassed diverse methodologies, including randomized controlled trials, cohort studies, and qualitative research.
Results: Findings from the systematic review demonstrated that midwifery continuity of care significantly improved the overall experience of infertile couples. It was associated with increased patient satisfaction, reduced stress and anxiety levels, and improved emotional well-being. The provision of consistent and personalized care by a dedicated midwife fostered a sense of trust, empowerment, and continuity throughout the infertility journey. Moreover, midwifery continuity of care resulted in enhanced communication and coordination between healthcare providers, leading to improved clinical outcomes and a higher likelihood of successful pregnancy.
Conclusion: In conclusion, endometriosis-induced infertility significantly impacts the psychological well-being of women and couples. Recognizing and addressing these psychological aspects is essential for comprehensive and patient-centered care. Interventions focusing on psychological support, counseling, and coping strategies can help individuals and couples navigate the emotional challenges associated with this condition. Collaborative efforts among healthcare providers, psychologists, and support networks are crucial for alleviating the psychological burden and enhancing the overall well-being of those affected.
Keywords: Continuity of Care, Couples, Infertility, Midwifery

Pnm-20: Psychological Aspects of Endometriosis-Induced Infertility: A Systematic Review

Taheri M*

Department of Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran
Email: tahery_mi@yahoo.com

Background: Endometriosis-induced infertility presents substantial challenges for women and couples aspiring to conceive. Beyond the physical implications, understanding the psychological impact of this condition is crucial. This systematic review aims to explore the psychological aspects associated with endometriosis-induced infertility and their implications for affected individuals and couples.
Materials and Methods: A comprehensive search was conducted across multiple electronic databases, including PubMed, Embase, and PsycINFO. Studies published between 2010 and 2022 were selected, focusing on the psychological aspects of endometriosis-induced infertility. Relevant articles were screened, and data extraction was independently performed by two reviewers. Various methodologies, such as quantitative surveys, qualitative interviews, and psychosocial interventions, were included.
Results: The review findings emphasize the profound psy-

chological consequences of endometriosis-induced infertility. Women facing infertility due to endometriosis often experience heightened levels of distress, depression, and anxiety. Feelings of grief, loss, and a sense of failure are common, stemming from the inability to conceive naturally. The psychosocial impact extends beyond the individual, affecting the well-being, dynamics, and quality of life of couples and relationships. Moreover, the review highlights the influence of endometriosis-related pain and symptomatology on psychological well-being. Chronic pain, coupled with the uncertainty surrounding fertility outcomes, contributes to increased stress and emotional strain. The lack of control over reproductive health and the invasive nature of medical interventions further exacerbates the psychological burden.

Conclusion: In conclusion, endometriosis-induced infertility significantly impacts the psychological well-being of women and couples. Recognizing and addressing these psychological aspects is essential for comprehensive and patient-centered care. Interventions focusing on psychological support, counseling, and coping strategies can help individuals and couples navigate the emotional challenges associated with this condition. Collaborative efforts among healthcare providers, psychologists, and support networks are crucial for alleviating the psychological burden and enhancing the overall well-being of those affected.

Keywords: Endometriosis, Infertility, Psychological Aspects, Psychosocial Impact

Pnm-21: An Overview of The Advantages and Disadvantages of Using A Deceased Donor in Uterus Transplantation

Taherkhani S

Department of Midwifery, School of Medicine, Arak University of Medical Sciences, Arak, Iran
Email: sakinehtaherkhani@yahoo.com

Background: In the context of increased demand for uterine transplantation and the possibility of a shortage of uterine grafts in the future, deceased donation can be a source for uterine graft. This study was designed to determine the advantages and disadvantages of using a deceased donor in uterus transplantation.

Materials and Methods: In this review study, the PubMed database was searched, with no time, language, or location restriction until February 1, 2023, using the words "uterine transplantation" or "uterus transplantation", "deceased donors", and "brain death donors".

Results: From 451 identified articles, 26 papers were entered the study for qualitative review. Based on the results, in deceased donation, the procurement of the uterus is faster and easier. The intraoperative and postoperative complications and adverse psychological consequences of donation do not threaten the donor. The recipient also does not experience adverse psychological consequences such as the burden of unpayable debt. However, the limited uterus evaluation of the donor before donation, the difficulty of coordinating medical teams and planning for surgery, the need for more logistics such as air transportation on permanent standby, longer waiting times for transplants, the high cost of transplantation and the increased possibility of bleeding after surgery in the recipient are among the disadvantages of using a deceased donor.

Conclusion: The lack of risk for the donor is the biggest advantage of using a deceased donor and the limited uterine evaluation of the donor before donation and the impossibility of planned surgery are the main disadvantages of using a deceased donor.

Keywords: Brain Death Donors, Deceased Donors, Review, Uterus Transplantation

Pnm-22: Clinical and Research Activities of The Oncofertility Task Force at ROYAN Institute

Yahyaei A*, Ghaffari F

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

Background: Oncofertility is a new concept that describes the communication network focused on medical methods to preserve reproductive function in cancer patients. Oncofertility refers to the relationship between oncology, reproductive medicine, sex therapy, pediatrics, and bioethics, and includes the risks associated with future fertility and solutions to minimize the effects of cancer treatment on fertility. Fertility preservation (FP) is an important issue in cancer patients and should be considered an important part of their cancer management. FP has been emphasized in international guidelines; in addition, sexual health and function should also be an essential part of treatment management of cancer patients who may be at risk for cancer treatment-induced infertility.

Materials and Methods: Despite the decades-long history of FP in cancer patients in the world, this field is about a decade old in Iran, where ROYAN institute is the most important active institution in the field. The gravity of the issue has led to the formation of the "Fertility Preservation in Cancer Patients Task Force" within this institute in 2017.

Results: The Task Force uses the world's latest, cutting-edge FP methods, including the oophoropexy, and the freezing of embryos, eggs, sperm, and ovarian and testicular tissue, to preserve fertility in adult men and women and immature girls and boys. It also pursues the mission of conducting relevant research projects aiming to improve the current treatment methods. The members of this specialized working group are divided into the clinical and research subgroups. The members of the clinical team are reproductive endocrinologist, reproductive urologist, oncologist hematologist, genetic counselor, psychologist, embryologist, advanced laparoscopist surgeon, anesthesiologist, pathologist, oncofertility nurse, ethic specialist, high risk pregnancy specialist, radiologist, and forensic specialist. The members of the research team are reproductive endocrinologist, reproductive urologist, oncologist hematologist, research embryologist, genetic specialist, stem cell & developmental biologist, epidemiologist, ethic specialist, research coordinator.

Conclusion: The services provided to adult women and immature girls in this research center comply with their pubertal

status and cancer treatment method.
Keywords: Oncofertility, Fertility Preservation, Cancer Patients, Royan Institute

Pnm-23: The Effect of Counseling on Treatment of Infertile Couples

Zare F[1*], Heidari Sarvestani M[2]

1. Department of Midwifery, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. School of Nursing and Midwifery, Isfahan University of Medical Sciences, Isfahan, Iran
Email: zare.fatemeh7575@gmail.com

Background: Infertility as a mental crisis has brought heavy stress on infertile couples and it threatens their mental health.96-48% of infertile couples have experienced psychological problems, and stress reduces their sexual function. The present study aims to investigate the effect of counseling and treatment of infertile couples. It was based on a review of studies.
Materials and Methods: This review study searched all the English articles indexed in PubMed, Google Scholar, Scopus Web of Sciences databases from 2017 until now with the keywords of infertility, counseling and infertility treatment.
Results: Potential tensions caused by treatments, especially hormonal drugs, cause high tension in individuals and couples. Also, patients under treatment have high anxiety about the failure of the treatment plan and low levels of adrenaline during egg retrieval have been reported to be associated with an increased chance of pregnancy. This shows that increasing anxiety levels shortly before egg retrieval may affect the ovulation phase. Mental problems have reduced their physical efficiency and their response to infertility medical treatments and on the other hand, the continuation of infertility and possible failures in the treatment stages lead to an increase in the psychological problems of these people.
Conclusion: Often physical treatment of infertility alone is not enough. Paying attention to the psychological needs of infertile couples is an essential part of successful infertility treatment. In fact, success in treating infertility is a combination of medical and mental health interventions and it should be considered part of the treatment process.
Keywords: Counseling, Infertility, Infertility Treatment

Pnm-24: The Role of Nutrition in Fertility

Zolfaghri F[2], Adib-Rad H[1*]

1. Department of Midwifery, Infertility and Reproductive Health Research Center, Health Research Institute, Babol, Iran
2. Department of Nursing and Midwifery, Student Research Committee, Faculty of Nursing and Midwifery, Babol, Iran
Email: AdibRad2015@gmail.com

Background: The nutrition has an important rule on infertility treatment. So we should improve the couple's information and knowledge about the effective nutrition on the infertility to increase the chance of fertility and decrease the negative emotional and mental effects of infertility on the couple.
Materials and Methods: Information from this survey with numerous articles from 2010 to 2023 in internet different sites and books collected and evaluated.
Results: Vitamin low deficiency and chemical poisons can intervene in ovum and sperm production and failure. Zink has been the widest nutritious element that has been studied on a couple's fertility improvement. Zink deficiency can cause some changes in the couple's chromosomes that decrease fertility and increase the rate of abortion. Also, the use of iron supplements causes a decrease in the rate of ovarian infertility dangerous meaningfully. Changing or replacing animal resources and protein with herbal resources, may cause a decrease in ovarian infertility. Food that is full of vitamin E like sunflower oil, fish liver oil and gourd seeds may cause an improvement on infertility. Essential fatty acids that exist in black raisin seeds oil, primrose oil, and Lenin seeds oil for gonadal normal function in both sexes are necessary. Also, Vitamin D can also prevent recurrent miscarriages.
Conclusion: Proper and enough nutrition must be the base of every disease like infertility. Therefore, nutritional support can help fertility and improve fertility methods.
Keywords: Fertility, Mineral, Nutrition

Authors Index

Royan Stem Cell Technology co.

Our Capabilities

1. Cord Blood Banking
2. Peripheral Blood Cryopreserving
3. Human Umbilical Cord Serum
4. Bone Marrow Storage
5. Producing Amniotic Membrane
6. Refinery Plant of Maternity Products(RPMP)
7. HLA Laboratory
8. Flow Cytometry Laboratory

www.RSCT.ir Telephone: +98(21) 27635000 Royan_cbb

Compare it...

IVF

Ideh Varzan Farda

CRYOTEB (Vitrification straw)

- Survival rate >90%.
- 10 colors available.
- The best Cooling and Warming Rates.
- Thin strip to allow high temperature exposure to specimens during vitrification and thawing.
- Diamond tip for an easy insertion into the cover straw.
- Identification marks to define the side where specimens should be loaded.

IUI CATHETER (Intrauterine insemination)

- 3 models: Simple, Precurved and Mandreled
- Rubberless syringe.
- Minimum dead space.

ET CATHETER (Embryo transfer catheter)

- 2 models: Simple, Echotip
- Minimum media required.
- Rounded tip eases passage through the cervix (o'bulb).
- Approximation marks on the inner catheter.

Ovum Aspiration Needle

- 2 models: Single lumen, Flushing line
- Echo tip enhances visualization of needle tip.
- Clean and smooth puncture minimizing trauma.
- Provide a continuous aspiration flow to minimize oocyte stress.
- Back-cut beveled.
- Flexible aspiration tube lines.

Micro-manipulation tools

- Micro-injection
- Holding
- Blastomere aspiration
- Polar body biopsy
- Zona drilling/assisted hatching
- Partial zona dissection

SDFA KIT (Sperm DNA Fragmentation Assay Kit)

- Measurement of sperm DNA fragmentation in easy and quick manner.
- No need of complex laboratory equipment.
- 50 reactions.
- Store at 2-8ºC for 8 months.
- Free analysis software.

DIFF QUICK KIT (Rapid Sperm Staining Kit)

- Used for evaluating the characteristics of human sperm morphology.
- 100 reactions.
- Store at 2-8ºC for 12 months.

SCMA KIT (Sperm chromatin maturation assay kit)

- Evaluate the DNA maturity of human sperm by staining sperm chromatin.
- 100 reactions.
- Store at 2-8ºC for 12 months.

LD Kit (Leukocyte detection kit)

- Elevated level of WBC in the semen is indicative of genital tract infections.
- 250 reactions.
- Store at 2-8 ºC for 6 months.

Vitality Assay KIT

- Used for evaluating the live sperm in human semen, verify the membrane structure and functional.
- 150 tests.
- Store at 2-8ºC for 6 months.

امیر آباد شمالی، خیابان شانزدهم، پارک علم و فناوری دانشگاه تهران، ساختمان شماره ۲

www.ivfco.ir + 98 9205445464 02186093832

masstec MEDICAL

شرکت مهندسی سلامت یار حکیم

- تولید کننده تجهیزات پزشکی دستگاهی و مصرفی
- در حوزه‌های زنان، باروری و گوارش با نشان تجاری Masstec
- دارای تاییدیه IMED و مجوز صادرات
- دارای تاییدیه از مراکز معتبر درمانی

FSC Free Sales Certificate | IMED National Medical Device Directorate IR.IRAN | جمهوری اسلامی ایران وزارت بهداشت، درمان و آموزش پزشکی | CSQ ISO 13485 | CSQ ISO 9001 | IQNet

Endometrial Suction Curette (PIPELLE)

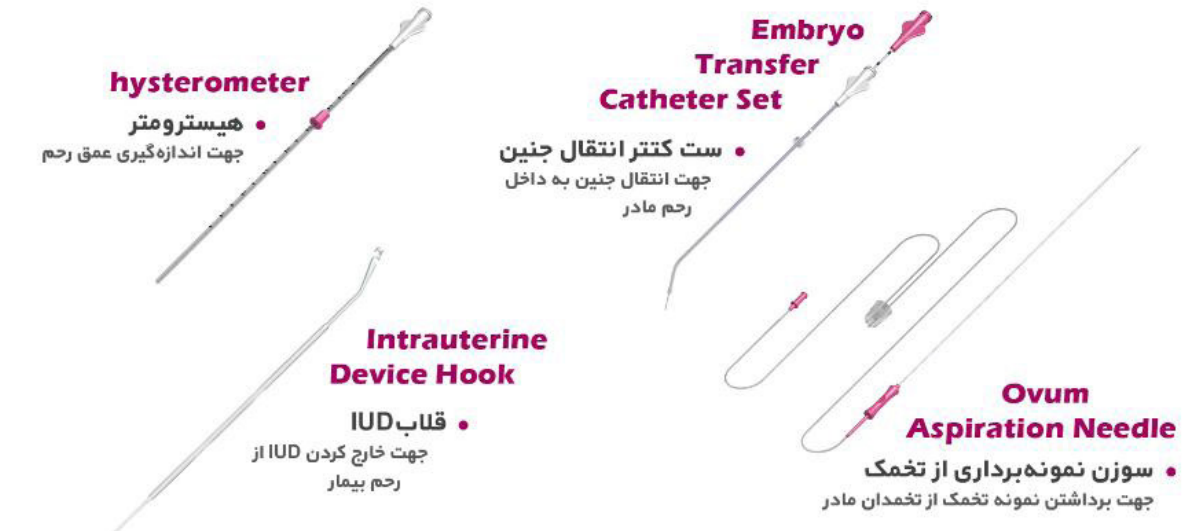
- **پایپل ساده**
  جهت نمونه‌برداری از اندومتر
- **پایپل سرنگ‌دار**
  جهت نمونه‌برداری از اندومتر
  جایگزینی مطمئن برای کورتاژ شخصی

IUI Catheter

- **کتتر IUI**
  جهت استفاده در بارورسازی بانوان

ساده

ماندرل‌دار



hysterometer

- **هیستروومتر**
  جهت اندازه‌گیری عمق رحم

Embryo Transfer Catheter Set

- **ست کتتر انتقال جنین**
  جهت انتقال جنین به داخل رحم مادر

Intrauterine Device Hook

- **قلاب IUD**
  جهت خارج کردن IUD از رحم بیمار

Ovum Aspiration Needle

- **سوزن نمونه‌برداری از تخمک**
  جهت برداشتن نمونه تخمک از تخمدان مادر

آدرس دفتر: اصفهان، خیابان هشت بهشت غربی، نبش چهارراه گلزار، ساختمان باران، طبقه دوم، واحد ۱۱۶

آدرس کارخانه: اصفهان، شهرک صنعتی رازی، فاز دوم، خیابان ششم، شرکت مهندسی سلامت یار حکیم

شماره تماس: ۴۴ - ۰۳۱۳۲۶۶۷۵۴۲

وب‌سایت: www.masstecmedical.com

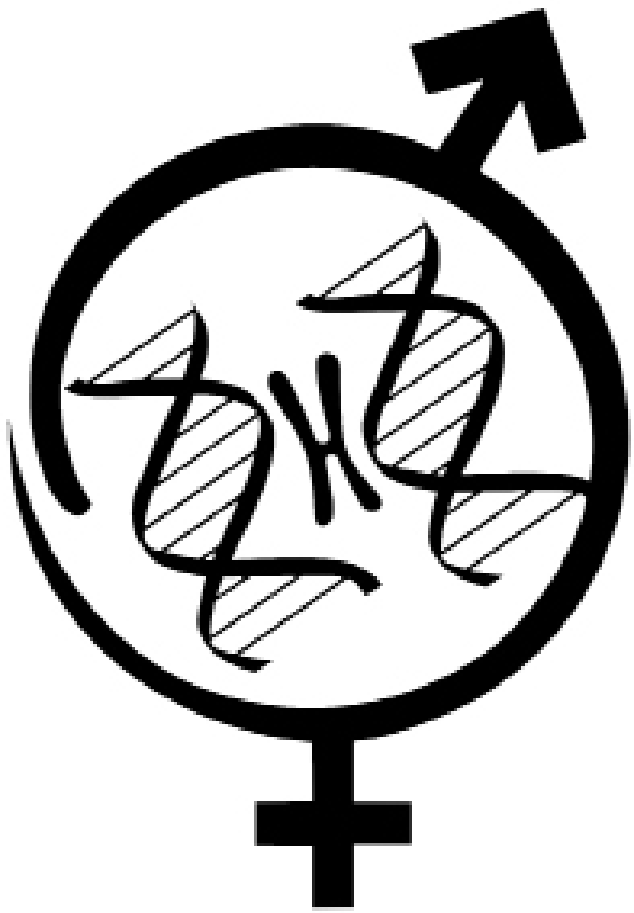
اینستاگرام: @masstecmedical

منتظر محصولات جدید ما باشید...

Abstracts of

Royan International Hybrid Twin Congress

19th Congress on Stem Cell Biology and Technology
29-30 August 2023



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Abstracts of the 19th Congress on Stem Cell Biology and Technology (2023)

Contents:

Congress President:
Shahverdi AH. Ph.D

Congress Chairperson:
Sattarian L., Ph.D

Committees
Organizing Committee:
Abdollahian E., MA
Afsharian P., Ph. D
Azimi R., M.Sc
Dadkhah F., M.Sc
Daliri L., M.Sc
Ezabadi Z., M.Sc
Fathi R., Ph.D
Lotfipanah M., M.Sc
Sheikhan M., MA
Sharif M.R., M.Sc
Taghiyar L., PhD
Vosough M, MD., Ph. D
Vosough Taghi Dizaj A., M.D
Zamanian MR., MD., Ph. D
Zoghi F., B.Sc

Executive Committee:
Faraji S.
Favayedi R.
Ghezelayagh ZA.
Jafarpour F.
Hosseini H.
Koohestani S.
Malekzadeh F.
Mirshekar Z.
Rashki L.
Roohbani Sh.
Saadat A.
Shajarehpour L.
Taheri E.

Scientific Board

Abroun S.
Adeli M.
Aghdami N.
Ajalloueian F.
Ali Moghaddam K.
Atari MA.
Attari F.
Babashah S.
Baghban Eslami Nejad M.
Bagheri F.
Baharvand H.
Bahrami AR.
Colom B.
Daemi H.
Dalirfardouei R.
Davaran S.
Ebrahimi M.
Esfandiari F.
Ezzatizadeh V.
Farokhi M.
Ghaedi K.
Ghanbarian H.
Ghanian MH.
Gharaati M.
Hadizadeh Shirazi N.
Haeri A.
Hajizadeh-Saffar E.
Hamidieh AA.
Hassani N.
Hay D.
Heydari S.
Hosseini S.
Imani R.
Jahan J.
Javan M.
Jaymand M.
Karamali F.
Kazemi M.
Kazemnejad S.
Kiani S.
Khang G.
Khoddami V.
Khoee S.
Larijani B.
Lengefeld J.
Mahmoudi T.
Malekzadeh R.
Mansouri K.
Mashinchian O.
Mohammadi A.
Moghaddam MM.
Moghaddasali R.
Mollapour Sisakht M.
Montazeri L.
Moradi Sh.
Mostafavi E.Mozafari M.
Nasiri Kenari A.
Nemati Sh.
Niknejad H.
Nussler A.
Pahlavan S.
Pezeshki-Modaress M.
Piryaei A.
Poorghadamiari H.
Pourfarzad F.
Rahimi Movaghar V.
Rajabi S.
Ramezani R.
Sadeghi H.
Sadeghian S.
Salehi M.
Sattarian L.
Shekari F.
Shahpasand K.
Shahzadeh Fazeli SA.
Sharifi Zarchi A.
Shiri Z.
Soleimani M.
Taghiyar L.
Tahamtani Y.
Totonchi M.
Trocoli Torrecilhas AC.
Vazquez-Armendariz AI.
Vosough M.
Warkiani M.

Congress Chairperson

Leila Satarian

As the scientific program chair, I welcome you to join to the "19th Royan International Congress on Stem Cell Biology and Technology (ICSCBT) will be held during 30-August to 2-December, 2023 in the beautiful city of Iran, Tehran. ICSCBT 2023 is an international platform to bring together innovative academics and industrial experts in the affiliated fields of Stem cell research and its innovative applications.
So, we are very honored to welcoming all of you to Royan congress 2023 at Iran, 2023.

Kind regards
Leila Satarian, Ph.D
Chairperson of 19th Royan International Congress
on Stem Cell Biology & Technology

Invited Speakers

Is-1: Delamination-Free Micro/Nanostructured Layered Scaffolds for Tissue Engineering Applications

Ajalloueian F

Department of Health Technology, IDUN center of excellence, Technical University of Denmark, Denmark
Email: faaj@dtu.dk

Is-2: Advances of Regenerative Medicine in Diabetes Therapy

Aghayan HR

Department of Medicinal Chemistry and Drug Applied Research Center, Tabriz University of Medical Sciences, Faculty of Pharmacy, Tabriz, Iran
Email: hraghayan@gmail.com

Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran Diabetes (DM) and its complications are big health challenges worldwide. Many therapeutic approaches have been suggested for DM but none of them are curative. Regenerative medicine (RM) is a promising approach to treating chronic and incurable diseases. This new branch of biomedical sciences has opened a new horizon to the treatment of DM and its complications. RM products can be classified into four categories: cell-based products, tissue-engineered products, gene therapy products, and cell derivate. Pancreatic islet transplantation is a cell-based therapy for diabetes that was done before the introduction of the RM concept. Newer strategies like mesenchymal stem cell transplantation, transplantation of islet progenitors or insulin-producing cells, and application of tissue-engineered skin substitutes are examples of promising regenerative therapies for DM and its complications. This presentation will review these new therapeutic advances for DM.

Is-3: Engineered Exosomes Carrying microRNAs to Induce Apoptosis in Cancer Cells

Arefian E

Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: arefian@ut.ac.ir

Glioblastoma multiforme (GBM) is an aggressive and lethal brain cancer, which is incurable with standard cancer treatments. miRNAs have great potential to be used for gene therapy due to their ability to modulate several target genes simultaneously. We found various miRNAs including mir-429, miR-424, miR-129, miR-4698, miR-548X, and miR-34a inhibit the cell cycle and induce apoptosis in glioblastoma cancer cells. They have several predicted target genes from the different signaling pathways that are analyzed using bioinformatics tools and confirmed by experimental methods including luciferase assay, Real-Time PCR, and western blot. Brain tumors, especially glioblastoma multiforme (GBM) treatment are still challenging due to the lack of efficient drug delivery systems. Using exosomes as nano-sized, immunologically silent, blood-brain-barrier permeable, and natural delivery tools is a game changer in delivering apoptosis-inducing agents to GBM. The engineered mesenchymal stem cells (MSCs) produced extra vesicles carrying anti-EGFRvIII (ab139) antibody on their surface while encapsulating Cytosine Deaminase (CDA) and microRNAs. The extra-vesicles were characterized for their size, morphology, protein content, and markers using dynamic light scattering and nanoparticle tracking analysis, cryoTEM, and Western Blotting. miR overexpression and Lamp2-ab139 protein expression were analyzed using real-time PCR and flow cytometry, respectively. The armed exosomes were delivered to EGFRvIII positive GBM cells as well as wild type cell line, which was EGFRvIII negative. Apoptosis was quantified using flow cytometry in both EGFRvIII negative and positive U87 cells, receiving one CDA or miR or a combination of them. Findings clearly show the effectiveness of drug delivery using engineered apoptosis-inducing exosomes and also reveal the improved apoptosis in the combination interference approach.
Keywords: Glioblastoma multiforme, microRNAs, Exosomes, Cytosine Deaminase

Is-4: Tumor Immunosuppressive Microenvironment in Breast Cancer: Focus on Exosome-Based Cell-To-Cell Communication

Babashah S

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: babashah@modares.ac.ir

The tumor microenvironment (TME) consisting of distinct cell types including stromal cells and immune cells has recently emerged as a pivotal player in tumor development and progression. Cancer-associated fibroblasts (CAFs), one of the major components of the tumor stroma, contribute to an immunosuppressive TME) through the induction and functional polarization of protumoral macrophages. Breast cancer (BC) is characterized by having a large population of tumor-associated macrophages (TAMs), most of which exhibit the M2 phenotype. Both CAFs and TAMs support tumor progression and an increased number of either is strongly associated with poor clinical outcomes. CAFs and TAMs do more than just reciprocal communication with the tumor cells; they also interact with each other in a dynamic way in the tumor milieu. Numerous studies have indicated that CAFs play a crucial role in monocyte recruitment and M2 polarization in different types of tumors, such as BC. Investigating TME-induced macrophage polarization and communication between TAMs and tumor cells is crucial for further understanding of TAM-related pro-tumor outcomes and the potential development of novel therapeutic strategies. Exosome-mediated transfer of functional coding and non-coding RNAs is a mechanism of genetic exchange between cells in the TME, thereby affecting tumor development and progression. Although CAFs are important in the formation of TME and in interacting with tumor cells, the effects of their secretome on the behavior of immune stromal cells, particularly in terms of tumor progression, require further investigation. From a therapeutic intervention perspective, intercellular communications mediated by exosomal miRNAs are attracting increasing attention due to their contributions to tumor progres-

sion by reprogramming the TME.

Is-5: Innovative Cell-Free Strategy Against Osteoarthritis: *In Vitro* And *Ex Vivo* Evidence of The Therapeutic Action of Asc-Secretome

Brini AT

Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy
Laboratory of Biotechnological Applications - IRCCS Ospedale Galeazzi-Sant'Ambrogio, Italy

Email: anna.brini@unimi.it

Osteoarthritis (OA) is a chronic condition affecting the musculoskeletal system and it represents one of the leading causes of disability worldwide. It is the most frequent degenerative joint disease and is still lacking a satisfying treatment able to delay and revert its course. Familiarity, mechanical insults, and obesity are among the risk factors, together with gender and age. Right now, several non-pharmacological and pharmacological strategies are considered to alleviate pain and reduce chronic inflammation.
Among novel therapies against OA, conditioned medium (CM) derived from Adipose Derived Stem Cells (ASCs) displays a promising therapeutic potential, and it could be a valid alternative to cell-therapy. ASC-CM is released by ASCs during *in vitro* culture and can drive regeneration. It includes soluble mediators and Extracellular vesicles (EV) with an endosomal or a plasma membrane origin and with different dimensions (small and large EVs). ASC-CM characterization indicates the presence of chemokines, cytokines, receptors, inflammatory mediators, growth factors and lipids involved in cell signaling and inflammation.
The therapeutic potential of ASC secretome has been shown in both 2D and 3D experimental models of OA: human primary articular chondrocytes (CHs) and human osteochondral explants both stimulated with TNFα.
In vitro, in human CHs TNFα induces a hypertrophic shift characterized by the aberrant expression of type X Collagen and the abnormal activity of matrix metalloproteinases (MMPs) efficiently blunted by ASC-CM treatment and restoring both hallmarks to normal levels.
In the human osteochondral explant model, ASC-CM reduces MMP activity, GAG production and the levels of several catabolic and inflammatory mediators induced by TNFα treatment.
In conclusion, the action of ASC-CM in blunting OA-related hallmarks suggests its potential as an innovative cell-free orthobiologic although further investigations are needed.

Is-6: Adipose-Derived Mesenchymal Cells and Their By-Products Promote Regeneration and Immunomodulation in Preclinical Experimental Models

Brini A

Department of Biomedical, Surgical and Dental Sciences - University of Milan, Milan, Italy
Laboratory of Biotechnological Applications - IRCCS Ospedale Galeazzi-Sant'Ambrogio, Milan, Italy
Email: anna.brini@unimi.it

Mesenchymal stem cells (MSCs) are isolated from several tissues and expanded *in vitro*. Adipose tissue is an attractive abundant source of MSCs (ASCs, adipose-derived stromal/stem cells) able to differentiate into cells of the mesodermal lineage such as osteoblasts, adipocytes, chondrocytes.
ASCs are not significantly affected by donor's age and are known to be immunomodulatory through the regulation of immune cells by mechanisms that include the release of soluble factors (secretome). Preclinical studies indicate the therapeutic role of ASCs both in regenerative medicine and autoimmune diseases and clinical trials have produced promising results.
Previously our research group showed the regenerative action of autologous ASCs by repairing Critical Bone and Osteochondral Defects in preclinical models. Next, due to ASCs immunomodulatory features and their by-stander action we decided to administer either them or their secretome (CM) in three preclinical murine models of neuropathic pain: sciatic nerve chronic constriction injury (CCI), experimental diabetic pain and chronic pain associated with induced osteoarthritis (OA).
hASCs and their conditioned medium (CM) reduced neuropathic pain symptoms such as hyperalgesia and allodynia for a long-lasting period of time. The ability of hASC and their secretome to modulate host cell response is exerted affecting cytokine levels in the nervous system. Interestingly, secretome analysis revealed 101 factors associated with immune regulation; and, hASC-CM effect correlates with its ability to reduce the neuroinflammatory condition in both the peripheral and central nervous system.
We suggest that hASC-CM is a valid treatment option for controlling nerve hypersensitivity, exerting a rapid and long-lasting pain relief. The mechanisms underpinning its effects are likely linked to the positive modulation of neuroinflammation in peripheral and central nervous system that sustains peripheral and central sensitization.

Is-7: The Extracellular Vesicle Biomolecular Corona

Buzas E

Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary
Email: edit.buzas@gmail.com

In the synthetic nanoparticle field, it has been known for a long while that a biomolecular corona is formed around the surface of nanoparticles in bodily fluids. However, only recently, evidence was provided for the formation of a protein corona around extracellular vesicles (EVs) in biological fluids. Interestingly, a naturally formed corona can be adsorbed onto the surface of EVs not only extracellularly but also within the releasing cells. Besides naturally formed protein coronas, EVs can be artificially stripped and re-decorated with adsorbed proteins. Furthermore, EVs or can be engineered to express exofacial ligand-binding molecules that ensure the formation of a specific corona around the surface of the vesicles. There is a growing body of evidence that the EV corona play important roles in physiological and pathophysiological functions. Moreover, modulation of the EV corona may alter detection and affinity capture of the vesicles. Importantly, like in the case of synthetic nanoparticles, a complex biomolecular corona can be found in association with EVs, which not only contains proteins but also nucleic acids and lipoprotein particles. Therefore, certain molecules, previously identified as contaminants of EV preparations, are now recognized as external cargo components of EVs. Although the recently recognized biomolecular corona adds

additional complexity to EVs, it also holds very important yet unexploited potential in diagnostics and therapeutics.

Is-8: NK Cell Therapy to Target Brain Tumors and Neuroblastoma Stem Cells

Faranoush M

Paediatric Growth and Development Research Centre, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
Email: faranoush47@gmail.com

Is-9: Understanding the Nano-Bio Interface: From Clinical Translation of Targeted Drug Delivery

Farokhzad OC

Seer, Inc. Reswood City, California, USA
Email: of@seer.bio

The two most used nanoparticles for biomedical applications are polymeric nanoparticles and lipid nanoparticles. Optimization of the physicochemical properties of nanoparticles for the desired in vitro and in vivo characteristics can accelerate their successful development and commercialization. Recent advances in understanding the nano-bio interface have translated to the development of more effective nanotherapeutics, nanodiagnostics and tools for rapid, deep and unbiased sampling of the proteome. The goal of this talk is to review our efforts in the design and optimization of nanoparticles, which formed the foundation for the clinical translation of two first-in-kind targeted nanotherapeutics and the first-in-kind commercial platform for scalable deep unbiased proteomics analysis, and to discuss the lessons learned in the development of these technologies

Is-10: ETEC Infection Control With Specific Polyclonal Antibody

Ghaedi S

Department of Animal Biotechnology, Reproductive Biomedicine Research Centre, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: Sirous.ghaedi@gmail.com

Introduction: Enterotoxigenic Escherichia coli (ETEC) is characterized by the ability to produce major virulence factors including colonization and enterotoxins, and some of ETEC cause recurrent and widespread calf diarrhea. Antibodies are an important component of the immune system, and fight infections as a part of the first line defense. The antibodies perform a range of beneficial functions for the host, including protection against pathogen colonization. Understanding the pathogenic mechanisms of ETEC and the interaction between the specific polyclonal (SpAb) antibody and ETEC represents an opportunity to control ETEC infection.
Materials and Methods: in this study we surveyed the growth inhibition and morphology changes of ETEC K99 with different doses of SpAb (Anti E.coli k99 IgY).
Results: Killing curves show that all dose of specific pAb and high dose of non-specific pAb inhibited the growth of ETEC k99. Moreover, the bacteriostatic effect was enhanced in stationary phase of bacterial growth and it is stable in a dose-dependent manner. Also, the MIC curve indicated that the high dose of specific pAb 93,8 % inhibition of growth ETEC k99 and this effect was not significantly different with the gentamicin MIC result (control positive).
Conclusions: Administration of specific polyclonal antibody supplements may reduce the risk of infections with ETEC, hence contributing to a reduction or a delay in the development of multi-resistant bacteria.
Key words: pAb, ETEC, MIC, diarrhea.

Is-11: The Role of Long Non-coding RNAs in Cardiomyocytes' Cell Cycle Arrest

Gharbi S

Department of Genetics, Shahid Bahonar University of Kerman
Email: magharbi@gmail.com

Cardiac regeneration is a major challenge of modern medicine due to cell cycle arrest, which happens in mature cardiomyocytes after birth. This process which specifically happens in mammals, has been less characterized in human. This emphasizes the importance of clarifying molecular regulators of this phenomena. Many regulatory factors including non-coding RNAs which govern this process, are still unknown to us and needs particular attention due to their high contribution to the genome (≥ 97%). Especially, long non-coding RNAs (lncRNAs), which perform their role through interaction with transcription factors (TFs), proteins or miRNAs, may address more detailed molecular mechanisms into the cardiomyocytes' cell cycle arrest. In this regard, co-expression analysis of lncRNAs/mRNAs or lncRNAs/TFs might provide important information on the mRNA and TFs, which are being regulated by lncRNAs during cell cycle arrest. We took the advantage of available RNA-seq datasets (PRJNA353755) and compared two different stages of cardiomyocyte development, including embryonic immature and adult mature cardiomyocytes from human biopsies. Differential analyses were applied using HTSeq count and Deseq2 to find deregulated genes of lncRNAs and mRNAs. We chose some top candidates for confirmation *in vitro*, which expression pattern were studied in human embryonic stem cells-derived cardiomyocytes (hESC-CM) at different stages of differentiation (Days 0, 7, 10, 20 and 40), which corresponded to immature and mature cardiomyocytes. Among studied lncRNAs, STARD4-AS1, H19 and LINC1 were downregulated when hESC-CM progressed toward mature phenotype. However, LINC2 was upregulated. MEIS1 and HOXB13 which are two TFs involved in cell cycle arrest, were upregulated as well. We also identified the biological network behind these deregulated lncRNAs. Altogether, these findings may help to unravel some unknown molecular mechanisms of cell cycle arrest, which could have a broad impact on the mechanistic studies of heart development as well as novel regenerative therapies.

Is-12: Molecular Gene Therapy, Scientific and Ethical Considerations in Engineering Embryos using CRISPR-Cas9

Golkar Zh

School of Health and Natural Science, Voorhees College, Denmark, SC, United States
Email: zhabizg@yahoo.com

Is-13: Base Editing to Combat Blindness in Wolfram Syn-

drome

Groef LD

Cellular Communication and Neurodegeneration Research Group, Biology Department, Leuven Brain Institute, KU Leuven, Belgium
Email: lies.degroef@kuleuven.be

Wolfram syndrome (WS) is an inherited childhood disease characterized by type I diabetes mellitus, diabetes insipidus, blindness, deafness, neurodegeneration and eventually early death, due to autosomal recessive mutations in the WFS1 (and rare WFS2) gene. The limited understanding of the exact role of WFS1 and the pathophysiology of WS co-account for the current lack of treatments for WS. As WS is due to a genetic abnormality in the WFS1 (or WFS2) gene, genetic correction might be the most effective way to treat the disease. This can be done via genome editing methods. The main objective of this work is to generate proof-of-concept that CRISPR base editing can be used to correct the WFS1 gene in the retina and/or optic nerve of a WS mouse model.
We recently demonstrated that CRISPR base editing is a very efficient tool (>80% of cells edited) to correct point mutations in WFS1 iPSCs (Nami et al. Crispr j 4, 502-18, 2021). In the meantime, we have proven that CRISPR base editing can also efficiently correct these mutations in non-dividing cells (including neurons, astrocytes and oligodendrocytes) and restore WFS1 expression levels. Furthermore, via mechanistic studies in WS patient iPSC-derived neurons and oligodendrocytes, we have been interrogating the underlying disease mechanisms and pinpointing which cell type must be targeted by the CRISPR base editing therapy. Using all these novel insights, we are now evaluating the efficiency and safety of CRISPR base editing therapy upon delivery in the mouse visual system. Thereto, we have created a transgenic knock-in mouse, harboring a point mutation that has been found in a WS patient. In this novel WS mouse model, besides testing the *in vivo* safety of this therapeutic approach, we will assess its efficiency via morphological and functional evaluations of visual system integrity.
This ongoing preclinical study is one of the first steps that may ultimately lead to the implementation of CRISPR-mediated base editing in somatic cells in the clinic. For now, we focus on the visual system, as it offers unique opportunities for directed drug delivery and *in vivo* monitoring of structure and function. While this approach is highly valuable to cure blindness in WS patients, in the future, the CNS as a whole will need to be targeted to cure WS.

Is-14: Mesenchymal Stem Cell Transplantation In Newly Diagnosed Type-1 Diabetes Patients: A Phase I/II Randomized Placebo-Controlled Clinical Trial

Hajizadeh-Saffar E

Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: en.hajizadeh@royan-rc.ac.ir

Type-1 diabetes (T1D) occurs following autoimmune-induced pancreatic beta cells death. Among several treatment modalities, mesenchymal stem cells (MSCs) transplantation is promising for autoimmune disorders due to immunomodulation, regeneration, and migration to damaged tissue upon systemic injection. This study assessed the safety and efficacy of intravenous injection of autologous bone marrow-derived MSCs in newly diagnosed T1D patients.
After receiving informed consent, 21 patients who met the study criteria were enrolled and randomly assigned to receive either MSCs or placebo. Each patient in the experimental group received two doses of MSCs and was followed for at least one-year post-transplantation.
The results have shown that this transplantation is safe and significantly reduces the number of hypoglycemic episodes. MSCs transplantation improved glycated hemoglobin (HbA1c), shifted serum cytokine patterns from pro-inflammatory to anti-inflammatory, increased the number of regulatory T-cells in the peripheral blood, and improved quality of life. Early transplantation of MSCs significantly improved HbA1c and C-peptide levels and shifted pro-inflammatory cytokines to anti-inflammatory cytokines. Also, exercise combined with MSCs transplantation improved glycemic and immunologic indices.
Taken together, autologous MSC transplantation is safe and effective, and its early transplantation is a promising treatment in newly diagnosed T1D children suffering from hypoglycemic episodes.

Is-15: Extracellular Vesicles in Autoimmune and Inflammatory Diseases

Hashemi SM

Department of Immunology, Scholl of Medicine, Shahid Beheshti of Medical Sciences, Tehran, Iran
Email: smmhashemi@sbmu.ac.ir

Is-16: Y Chromosome Genes May Play Roles In The Development of Neural Rosettes From Human Embryonic Stem Cells

Khani F

Department of Stem Cell and Regenerative Medicine, Institute of Medical Biotechnology, National Institute of Genetic Engineering & Biotechnology (NIGEB), Tehran, Iran
Email: farzanehkhani61@gmail.com

Background: The human Y chromosome harbors genes that are mainly involved in the growth, development, sexual dimorphism, and spermatogenesis process. Despite many studies, the function of the male-specific region of the Y chromosome (MSY) awaits further clarification, and a cell-based approach can help in this regard.
Results: In this study, we have developed four stable transgenic male embryonic stem cell (ESCs) lines that can overexpress male-specific genes HSFY1, RBMY1A1, RPS4Y1, and SRY. As a proof of principle, we differentiated one of these cell lines (RPS4Y1 over-expressing ESCs) to the neural stem cell (rosette structure) and characterized them based on the expression level of lineage markers. RPS4Y1 expression in the Doxycycline-treated group was significantly higher than control groups in transcription and protein levels. Furthermore, we found Doxycycline-treated group had a higher differentiation efficiency than the untreated control groups.
Conclusions: Our results suggest that the RPS4Y1 gene may play a critical role in neurogenesis. Also, the generated transgenic ESC lines can be widely employed in basic and preclinical studies, such as sexual dimorphism of neural and cardiac

functions, the development of cancerous and non-cancerous disease models, and drug screening.
Keywords: Y-Chromosome Human Proteome Project (Y-HPP); human Embryonic Stem Cell (hESC); RPS4Y1; Stable cell line; Neural differentiation.

Is-17: Co-Delivery of Doxorubicin and Paclitaxel Via Noisome Nanocarriers Attenuates Cancerous Phenotypes in Gastric Cancer Cells

Khannazer N

Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: nikookhannazer@gmail.com

Gastric cancer (GC) is known as a deadly malignancy all over the world, yet none of the current therapeutic regimens have achieved efficacy. this current study has aimed to optimize and reduce treatment doses and overcome multidrug resistance in GC by developing optimum niosomal formulation for the delivery of doxorubicin (DXR), paclitaxel (PTX), and their co-delivery. The particles' size, polydispersity index (PDI), and entrapment efficacy (EE%) were optimized using statistical techniques, i.e., Box-Behnken and Central Composite Design. In contrast to soluble drug formulations, the release rate of medicines from nanoparticles were higher in physiological and acidic pH. Niosomes were more stable at 4 °C, compared to 25 °C. The MTT assay revealed that the IC 50 of drug-loaded niosomes was the lowest among all developed formulations. The apoptosis-related genes (CASPASE-3, CASPASE-8, and CASPASE-9) and tumor suppressor genes (BAX, BCL2) were evaluated in cancer cells before and after treatment. In comparison to control cells and cells treated with soluble forms of DXR and PTX, while the expression of BCL2 decreased, the expression of BAX, CASPASE-3, CASPASE-8, and CASPASE-9 was enhanced in cells treated with drug-loaded niosomes. Drug-loaded niosomes inhibited colony formation capacity and increased apoptosis in human AGS gastric cancer cells. Our results indicate that co-delivery of DXR and PTX-loaded niosomes may be an effective and innovative therapeutic approach to gastric cancer.

Is-18: Engineering Approaches for Reducing Foreign-Bobdy Response to Implanted Biomaterials

Kazemi Ashtiani M

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

Implanting biomaterials in the human body triggers an inflammatory and subsequent fibrotic process known as the foreign body response (FBR), which poses a substantial challenge for the long-term functionality and stability of medical devices, especially in the field of tissue engineering. In recent years, extensive research has been dedicated to exploring engineering approaches aimed at mitigating the effects of FBR in tissue engineered scaffolds. This presentation will begin by providing an overview of the underlying mechanisms of FBR, with a particular emphasis on the critical role played by surface interactions between implanted biomaterials and host tissues. The focus of this talk will be on a range of approaches that include surface coating, chemical modification using anti- fouling groups, and incorporation of bioactive molecules. By presenting these strategies, the aim is to shed light on innovative approaches that hold significant promise for enhancing the biocompatibility and long-term performance of tissue engineered scaffolds in clinical applications.

Is-19: Islet Encapsulation for Type I Diabetes

Liu W

Key Laboratory of Textile Science & Technology, Ministry of Education, College of Textiles, Donghua University, Shanghai 201620, China
Email: wjliuok@dhu.edu.cn

Encapsulation and transplantation of insulin-producing cells offer a promising curative treatment for type 1 diabetes (T1D) without immunosuppression. However, biomaterials used to encapsulate cells often elicit foreign body responses, leading to cellular overgrowth and deposition of fibrotic tissue, which in turn diminishes mass transfer to and from transplanted cells. Meanwhile, the encapsulation device must be safe, scalable, and ideally retrievable to meet clinical requirements. Here, I will report a durable and safe nanofibrous device coated with a thin and uniform, fibrosis-mitigating, zwitterionically modified alginate hydrogel for encapsulation of islets and stem cell-derived beta (SC-β) cells. The device is designed with a configuration that has cells encapsulated within the cylindrical wall, allowing scale-up in both radial and longitudinal directions without sacrificing mass transfer. Due to its facile mass transfer and low level of fibrotic reactions, the device supports long-term cell engraftment, correcting diabetes in C57BL6/J mice with rat islets for up to 399 days and SCID-beige mice with human SC-β cells for up to 238 days. In addition, we demonstrated the scalability and retrievability in dogs. Our results suggest the potential of this new device for cell therapies to treat T1D and other diseases.

Is-20: Mesenchymal Stem Cell-Based Immunotherapy for Solid Tumors

Ma B

School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China
Email: binma@sjtu.edu.cn

Immunotherapies represented by chimeric antigen receptor (CAR)-T cells and immune checkpoint blockade have been applied to the treatment of many cancers, but their efficacy is not ideal in most solid tumour patients. The main obstacles include poor lymphocyte infiltration in solid tumours and toxicity associated with systemic administration of drugs.
Mesenchymal stem cells (MSCs), in addition to their multidirectional differentiation potential and certain immune regulatory functions, are also used as drug vehicles due to their high availability, ease of cultivation and modification *in vitro*, and low immunogenicity. Our study confirmed the active migration of MSCs into tumours in mouse models, without enrichment in organs such as the liver, spleen, lung, and kidney. By delivering chemokine CXCL9 and immune costimulatory ligand OX40L/TNFSF4 via mesenchymal stem cells to the tumour site, we

achieved the goal of simultaneously recruiting and activating effector lymphocytes in the tumour. This strategy effectively suppressed the growth of subcutaneous tumours, and demonstrated potent therapeutic effects in a spontaneous colorectal cancer model that did not respond to immune checkpoint inhibitors. In addition, the MSC-based treatment can also improve the antitumor efficacy of PD-1 blockade and inhibit the growth of MHC-I deficient subcutaneous tumours. In subsequent studies, we compared the therapeutic efficacies of three TNF superfamily (TNFSF) costimulatory ligands overexpressed in MSCs in lung and colorectal cancer models, and observed even better antitumor effects from TNFSF9 and 18.
Our immunotherapeutic strategy using MSCs as vehicles avoids systemic toxic side effects and breaks the bottleneck of poor lymphocyte infiltration into solid tumours. Moreover, it can improve the efficacy of other immunotherapies for solid tumours.

Is-21: Collagen-based nanocomposite hydrogels in tissue engineering

Mirzaei E[1, 2*], Takallu S[1]

1. Department of Medical Nanotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
2. Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Emails: @sums.ac.ir

Collagen-based hydrogels are appealing biomaterials for tissue engineering and regenerative medicine because they are hierarchically organized materials that more accurately mimic the essential characteristics of biological tissues. However, there are limitations to their use, one of the main drawbacks is that the collagen hydrogels have low mechanical strength, which can limit their use in load-bearing applications. Additionally, the other limitation of collagen hydrogels is the absence of antimicrobial properties for tissue which can be a concern, as infections can compromise the success of tissue engineering procedures. Despite these limitations, collagen hydrogels remain a promising biomaterial for tissue engineering. Our researches are focused on developing novel methods to overcome these limitations and create new properties in collagen hydrogel. One of the best approaches for improving collagen hydrogel properties is the development of nanocomposite hydrogels. Our team conducted projects to develop collagen hydrogels with new properties. Some of the research projects that had positive in-vitro and in-vivo outcomes are the development of nanocomposite hydrogel of collagen/hydroxyapatite for use in bone tissue repair, collagen/silver nanoparticle for burn wound healing, collagen/graphene oxide nanoparticle for use in repairing nerve tissue, collagen/hyaluronic acid/cellulose nanocrystal for use in repairing cartilage tissue, and collagen/cellulose nanocrystals for use in repairing corneal tissue.
Keywords: Collagen hydrogel, Tissue engineering, Regenerative medicine, Nanoparticle, Nanocomposite hydrogels

Is-22: Challenges in The Translation of Stem Cell Research for The Development of New Cell-Based Therapies for Osteoarthritis

Mobasheri A

Research Unit of Health Sciences and Technology, Faculty of Medicine, University of Oulu, FI-90014, Oulu, Finland
Email: Ali.Mobasheri@oulu.fi

This presentation focuses on the translation of stem cell research for the development of novel and efficacious cell-based therapies for osteoarthritis (OA), the most common form of arthritis globally, for which there are no effective therapies. The translation of stem cell research for the development of new cell-based therapies for OA faces several major challenges. This lecture will outline some of the key challenges, including safety, efficacy, long-term outcomes, standardization, quality control, immunogenicity and immune response, ethics, regulatory issues, cost accessibility and acceptance by the public and healthcare systems. Addressing these challenges requires collaborative efforts among researchers, clinicians, regulatory agencies, and the public to ensure the responsible and successful translation of stem cell research for OA and related musculoskeletal conditions.

Is-23: Elimination of Tumorigenic Stem Cells for Safer Cell Therapy

Moradi Sh

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sharif.moradi@gmail.com

Is-24: Suicide Gene Therapy Using Allogeneic Adipose Tissue-derived Mesenchymal Stem Cell Gene Delivery Vehicles in Recurrent Glioblastoma Multiforme: A First-In-Human, Dose-Escalation, Phase I Clinical Trial

Oraee-Yazdani S

Department of Neurosurgery, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences
Email: saeed_o_yazdani@sbmu.ac.ir

Is-25: The Role of Long Non-Coding RNAs in Cardiac Fibrosis

Pahlavan S

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sarah.pahlevan2007@gmail.com

Background: Heart injuries from multiple causes can result in pathological remodeling and fibrosis, which in turn promote the development of heart failure. An acute myocardial infarction leads to an increased proliferation of cardiac fibroblasts (CFs) and the transition to the myofibroblast phenotype. During this fibrotic phase, myofibroblasts begin to secrete elevated levels of collagens and other ECM proteins. Moreover, fibrogenic growth factors such as transforming growth factor-β, cytokines including tumour necrosis factor-α, interleukin (IL)-1 and IL-6, and neurohumoral pathways trigger fibrogenic signalling cascades, which further promote fibrosis. In this study, we aimed to establish an *in vitro* cardiac fibrosis model to provide a platform for mechanistic studies at transcriptome and proteome level.
Materials and Methods: Human CFs were isolated from myocardial biopsies obtained during mitral valve replacement. The

biopsies were cut into 1 mm^2 pieces and explanted on the gelatin-coated plates. Explants were cultured overnight in FBS and thereafter, for one week in fibroblast culture media. Fibrosis induction was performed by employing three concentrations of Doxorubicin (DOX; 0.1, 0.25 and 0.5 μM) for 72 hours, and it was assessed by immune-staining of smooth muscle actin (α-SMA) and analysis of secreted collagen content. Real-time qRT-PCR was performed for validation of some candidate fibrosis-related long non-coding RNAs, which were obtained by bioinformatics analysis of available datasets.

Results: Isolated fibroblasts expressed CF-specific proteins; Vimentin, Collagen a1 and CD90. Treatment with 0.1 and 0.25 μM of DOX did not significantly affect cell viability or cell apoptosis, whereas it provoked fibroblasts activation. Myofibroblast induction was confirmed by staining against α-SMA, which is a fibrosis-related biomarker. Furthermore, DOX-treated cells secreted more collagen into the culture medium. The expression pattern of candidate fibrosis-related long non-coding RNAs LINC01618، PICART1, CA3-AS1, MYOSLID ،BANCR and DIO3OS, was confirmed in *in vitro* model of fibrosis.

Conclusion: The *in vitro* model of cardiac fibrosis can be served as a platform to study the underlying mechanisms of cardiac fibrosis, to evaluate the fibrosis-related transcriptional changes, and to develop novel therapeutic strategies.

Keywords: Cardiac fibrosis, Cardiac fibroblast, Myofibroblasts, Doxorubicin, Disease modeling

Is-26: Embryological Coordinates of Human Pluripotency

Pera M

Australian Institute for Bioengineering and Nanotechnology, University of Queensland, Stem Cells Australia Program Leader Australia
Email: Martin.Pera@jax.org

There is currently great interest in the potential of stem cell-derived embryo models to yield new insight into human development and its disorders. In order to derive these models and interpret the outcomes of experiments using them, it is important to have a clear understanding of the developmental coordinates of the cells from which they are built. The self-renewing subpopulation of human pluripotent stem cells grown under conventional culture conditions (activin/Nodal and FGF signaling) most closely corresponds to the early post-implantation epiblast of the primate embryo, a cell in the formative state of pluripotency that is capable of differentiation into all somatic lineages, the germ line, and extraembryonic tissues. In this lecture, we will discuss the molecular characterization of this cell population, the factors that control its stability, and its differentiation potential.

Is-27: Current Status of Targeted Immunotherapy for Cancer

Ranjbar A

Institute of Interventional Allergology and Immunology, Bonn/Cologne, Germany
Email: alireza.ranjbar@web.de

The cancer immunotherapy is regarded as a great hope in medicine. But despite the impressive successes that have been achieved, especially in melanoma and advanced lung cancer, this field of medicine is still facing challenges.

Just a few years ago, immunotherapy in the fight against cancer had little success. The idea of using our own immune system to fight cancer cells is more than a hundred years old. In 1867, the German surgeon Wilhelm Busch placed a woman suffering from cancer in the empty bed of a patient with erysipelas in the university clinic in Bonn. A short time later, the life-threatening tumor in the woman's throat shrank.

However, it was only in the 21st century that the diverse interactions between cancer cells and the immune system were sufficiently studied to break new ground in cancer therapy.

Mobilizing immune systems against cancer in order to generate long-lasting success is the focus of current research.

In addition to the activation of T cells by checkpoint inhibitors and bispecific T cell activating antibodies, the approach of adoptive T cell therapy is another promising strategy.

Here, T cells are removed from the patient's body to equip them *ex vivo* with tumor-specific T cell receptors so that they can act effectively against the cancer cells.

Also promising are chimeric antigen receptor (CAR) therapies, in which peripherally removed T cells from patients are genetically modified with a chimeric antigen receptor and the modified T cells are then returned to the patient's body.

There, the modified T cells "search" for the corresponding antigen, e.g. the antigen CD19, which is carried by most B cell malignancies, in order to dock and destroy them.

In several anti-CD19-CAR T-cell transfer therapy studies, a remission rate of 30-90% was achieved in patients with acute pre-B-cell leukemia (ALL), chronic lymphocytic leukemia (CLL) or B-cell malignancies.

The challenges of cancer medicine are not only aimed at developing new medicines, it is also being investigated whether the combination of immunotherapies with conventional treatment methods, such as radiation and chemotherapy or the coupled application of two immunotherapies, can lead to sustainable treatment successes can be improved.

However, many details about the complex mechanisms of the immune system still have to be learned at the molecular level before most types of cancer can be effectively combated with the body's own defences.

This article attempts to provide an overview of the current status of targeted immunotherapy for cancer.

Is-28: Blastoids: Modeling Mouse and Human Blastocyst Development and Implantation with Stem Cells

Rivron N

Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna Biocenter, Vienna, Austria
Email: nicolasrivron@gmail.com

The blastocyst is the early mammalian organism before implantation. We discovered how to promote the self-organization of stem cells intostructures remarkably resembling the mouse and the human blastocyst,which we called blastoid. Blastoids aremorphologically and transcriptionally similar to the blastocyst and containanalogs of all three cell types that further develop into the completeorganism (embryonic and extra-embryonic tissues). Because blastoidsmodel the pre-implantation stage, they can be introduced into the uterus(mouse model) or combined with uterine cells *in vitro* (human model) torecapitulate aspects of the hidden processes of implantation. Contrary toblastocysts, blastoids come in large numbers and facilitate the

moresystematic modulation and analysis of the impact of cell numbers, states,and communication mechanisms on development. As such, they representboth a technical and ethical alternative to the use of embryos forresearch. Using this approach, we investigate the multicellular interactionrules underlying blastocyst development and implantation in order totackle global health problems of fertility decline, family planning, and thedevelopmental origin of health and diseases.

Is-29: Tissue Engineering Strategies for Enhancing Cardiac Regeneration of Bone Marrow-derived Mesenchymal Stem Cells

Salim A

Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, 75270, Pakistan
Email: asmat.salim@iccs.edu

Objective: Tissue engineering, using biomaterials and stem cells, is a highly promising strategy for cardiac repair. Cardiac differentiation potential of mesenchymal stem cells (MSCs) can be enhanced by epigenetic modifiers. The study objective is to analyze the effect of zebularine, a DNA methyl transferase inhibitor, on MSCs seeded on a 3D collagen scaffold for cardiac differentiation upon *in vivo* transplantation.
Materials and Methods: Rat bone marrow MSCs were characterized and seeded on collagen scaffold and treated with 3 μM zebularine. Cytotoxicity analysis was performed to confirm the non-cytotoxic effect of the treatment. For *in vivo* analysis, rat myocardial infarction (MI) model was developed and zebularine treated MSC-seeded scaffold was transplanted and compared with the MI group, along with other groups in which only collagen scaffold and untreated MSC-seeded scaffold were transplanted. Cardiac function was assessed by echocardiography and histological analysis.
Results: Zebularine treatment in the 3D environment significantly enhanced cardiac differentiation of MSCs both at gene and protein levels. Substantial improvement in cardiac function was observed in the zebularine treated MSC-seeded scaffold group in comparison to the MI control. Histological analysis showed reduction in the fibrotic scar, improvement in left ventricular wall thickness and preservation of ventricular remodeling in the treated group. Immunohistochemical analysis revealed significant expression of cardiac proteins in the DiI labeled transplanted cells and number of blood vessels in the zebularine treated group.
Conclusion: It can be concluded that combination of 3D collagen scaffold with zebularine enhances cardiac differentiation potential of MSCs when transplanted in the myocardium of the rat MI model. Results of this study will provide an effective therapeutic strategy for improved cardiac regeneration in the clinical settings in future.

Is-30: From Bench to Bedside: Pioneering Stem Cell-Based Therapy for Parkinson's Disease

Schweitzer J

Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, USA
Email: JSCHWEITZER1@mgh.harvard.edu

Parkinson's disease is the second most common degenerative disease of the brain, and as the world population ages its prevalence increases. In the mid 20th century, breakthroughs in our understanding of the cause of this disease allowed the development of both surgical and medical treatments that significantly benefitted patients. Nonetheless, these treatments are aimed only at the symptoms and do not slow or reverse the cause of the disease. They are also limited by significant risks and side effects. Recognition that loss of dopaminergic cells in the midbrain is closely tied to the motor symptoms of the disease led to early efforts to replace these cells using various sources such as human fetal neurons, but such sources also carried significant limitations and concerns. The advent of stem cell technologies such as hESC and hiPSC opened a new era of possibilities for cell therapy in Parkinson's, including the use of a patient's own cells to create replacements for the lost dopaminergic neurons. Here we describe our work resulting in the first use in a human patient of this type of personalized, autologous cell therapy, and describe some of the current and future challenges to making this therapy a practical treatment option.

Is-31: Earlier Detection of Alzheimer's Disease Based on A Novel Biomarker cis P-tau by A Label-Free Electrochemical Immunosensor

Shahpasand K

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

Is-32: Conditioned Medium Derived EVs: Opportunities and Challenges

Shekari F

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

Cell culture-derived conditioned medium-derived extracellular vesicles (CCM-EV) are a common source of EV for therapeutic applications. Unmodified CCM-EV have the potential to function as "intrinsic" or "native" therapeutics, conveying the beneficial traits of the parent cell. In contrast, modified EVs can be generated from engineered cells or subjected to post-production alterations to accentuate or introduce therapeutic properties. CCM-EV also exhibits several benefits over parent cells, including resilience to various storage temperatures, susceptibility to strategies for maintaining function, and absence of replicative capability. However, GMP compatible approach for therapeutic CCM-EV isolation has been a matter of debate in recent years. Following optimizing CCM-EV isolation, the efficiency of this specific subpopulation of CCM-EVs has been checked in various in vitro tests, animal models of different diseases including brain injury, diabetes, premature ovarian failure, and Ashermann syndrome as well as clinical trial on COVID-19 patients. Our extensive results provide hopes for industrialized CCM-EVs isolation in GMP systems.

Is-33: Laboratory for Innovative Microtechnologies and Biomechanics (LIMB)

Tamayol A

Department of Biomedical Engineering University of Connecticut Health Center
Email: atamayol@uchc.edu

Conventional practices for the treatment of commonly observed medical problems such as chronic wounds and musculoskeletal injuries have shown limited effectiveness. To address this unmet need, micro- and nanoscale technologies are increasingly used. Bioprinting has emerged as a promising tool for generating scaffolds and cellular structures with rationally designed architectures. The new generation of bioprinters are looking at bioprinting directly inside the patients body. During this presentation, I will highlight our research progresses in developing solutions for the treatment of skin defects and volumetric muscle and bone loss. The presented microengineered platforms will have broad applications in the fields of tissue engineering, drug delivery, and drug testing.

Is-34: Positional Memory and Its Role in Limb Regeneration

Tanaka E

Senior Group Leader, Research Institute for Molecular Pathology, Vienna, Austria
Email: elly.tanaka@imp.ac.at

The salamander limb regenerates only the missing portion, and this biological phenomenon that relies on a property called positional memory in the limb. Cells on the proximal-distal and the anterior posterior axis of the mature limb show positional memory. Upon regeneration, cells that populate the distal blastema must lose this identity to build lower limb parts. We have been investigating the chromatin basis of positional memory. Along the proximal-distal axis we have observed differential occupancy of homeodomain protein encoding genes by modified histones that corresponds to the differential expression of Hox genes in upper arm versus hand amputations. Chromatin profiling has also identified evidence regeneration-specific enhancers that become accessible early in the process while developmental enhancers reappear at later stages.

Is-35: Biobanking of Cardiovascular Progenitor Cells; An Emerging Step Towards Biomedical

Vahdat S

Research Sadaf Vahdat Applied Cell Sciences Division, Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: s_vahdat@modares.ac.ir

Cardiovascular diseases (CVDs) are considered the leading causes of death worldwide and are estimated to increase by the aging of populations. Accordingly, lots of cell-based experimental and clinical studies have been conducted to study CVDs more precisely and to figure out how to cure patients; in this regard, distinct types of cells have been used ranging from stem cells to progenitors and fully differentiated cells. Among them, cardiovascular progenitor cells (CPCs), which are cardiac-committed proliferative cells with the potential to differentiate into almost all cardiovascular lineages, are introduced as promising cell sources for both experimental and clinical studies. CPCs can be isolated from myocardial tissues or be generated in vitro through cardiogenic differentiation of human pluripotent stem cells (hPSCs); however, large-scale expansion and accessibility of CPCs are required for their wide range of applications. Therefore, establishment of a culture condition for scalable expansion, maintenance and biobanking of (hPSC-derived) CPCs is highly demanded. Here, we report the results of our studies carried out to develop simple, and reproducible chemically-defined culture conditions by the chemical screening of signaling factors for the maintenance and storage of CPCs. Expanded CPCs retained their morphology, gene expression pattern, chromosomal stability, and in vitro differentiation potential over subsequent passages. CPCs could be cryopreserved successfully and expanded in carrier-free suspension culture condition, appropriate for their biobanking and scalable culture, respectively. Moreover, transplantation of expanded CPCs into infarcted hearts of rat models exhibited the safety and cardioprotective effects of administration of these cells. Expanded CPCs and their derivatives have been used in some commercialization and drug screening studies and might be promising cell sources for other types of experimental, developmental, tissue engineering, and cell-based clinical studies.

Is-36: Novel Molecular Therapies in Liver Cancer

Vosough M

Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Emial: masvos@yahoo.com

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-associated death. Hepatocarcinogenesis involves numerous processes, including the Sonic Hedgehog (SHH) signaling pathway and ER stress, which participate in the initiation, progression, migration, recurrence, and maintenance of HCC cancer stem cells. Furthermore, SHH signaling and ER stress regulates various cellular behaviour such as proliferation, differentiation, cell survival, self-renewal, and epithelial-mesenchymal transition (EMT). Glioma-associated oncogene family zinc finger (GLI) and XBP1 are transcription factors playing important physiological roles in the development of many organs and are deregulated in cancer. In this study, we highlighted the importance of GLI and XBP1 transcription factors on cancerous phenotype of Huh-7 cells using Decoy oligodeoxynucleotide (ODN) and inhibited GLI/XBP1 binding to the promoters of downstream genes. GLI-specific/XBP1 Decoy ODNs were transfected into Huh-7 cells and the transfection efficiency was measured using fluorescent microscopy. Next, the effects of GLI-specific/XBP1 Decoy ODN transfection on the Huh-7 cells' cancerous phenotypes were assessed, in terms of apoptosis, cell viability, proliferation rate, colony formation, and migration capacities. Furthermore, the expression level of genes associated with the SHH signaling pathway and ER stress were assessed using quantitative real-time polymerase change reaction (qRT-PCR). In this study, it is demonstrated that inhibition of SHH signaling pathway/XBP1 using GLI-specific Decoy ODN led to a decline in the growth rate and progression of HCC cells, decreased migration capacity, and attenuated EMT progression. According to these data, it could be supposed that inhibition of the SHH pathway/ER stress using GLI/XBP1-specific Decoy ODNs, in combination with established medical settings, could be considered as a new potential therapeutic ap-

proach in HCC.
Keywords: Hepatocellular carcinoma, Decoy oligodeoxynucleotide, Glioma-associated oncogene (GLI), ER stress, XBP1, Sonic Hedgehog signaling pathway

Is-37: Manipulation of Gut Microbiom and Risk of Breast Cancer Later in life

Yasavoli H

Cellular and Molecular Medicine Department, Faculty of Medicine, University of Ottawa, Ottawa, Canada
Email: hyasa068@uottawa.ca

I-38 Generation and Functional Characterization pf PLAP CAR-T Cells Against Cervical Cells

Yekehfallah V

1. School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: vahidyekkefallah@gmail.com

Chimeric antigen receptor (CAR) T-cell therapy is one of the cancer treatment modalities that has recently shown promising results in treating hematopoietic malignancies. However, one of the obstacles that need to be addressed in solid tumors is the on-target and off-tumor cytotoxicity due to the lack of specific tumor antigens with low expression in healthy cells. Placental alkaline phosphatase (PLAP) is a shared placenta- and tumor-associated antigen (TAA) that is expressed in ovarian, cervical, colorectal, and prostate cancers and is negligible in normal cells. In this study, we constructed second-generation CAR T cells with a fully human scFv against PLAP antigen and then evaluated the characteristics of PLAP CAR T cells in terms of tonic signaling and differentiation in comparison with delta PLAP CAR T cells and CD19 CAR T cells. In addition, by co-culturing PLAP CAR T cells with HeLa and CaSki cells, we analyzed the tumor-killing functions and the secretion of anti-tumor molecules. Results showed that PLAP CAR T cells not only proliferated during co-culture with cancer cells but also eliminated them in vitro. We also observed increased secretion of IL-2, granzyme A, and IFN-gamma by PLAP CAR T cells upon exposure to the target cells. In conclusion, PLAP CAR T cells are potential candidates for further investigation in cervical cancer and, potentially, other solid tumors.

Oral Presentation

Os-1: Nerve Regeneration and Functional Recovery in a Rat Sciatic Nerve Injury Model Using Schwann Cells and Polyacrylonitrile Conduit

Bostani A[1*], Hoveizi E[2]

1. Department of Plant, Cell and Molecular Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
2. Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
Email: Aliasgharbostani98@gmail.com

Objective: Peripheral nerve injuries are a prevalent type of trauma to the nervous system. The goal of treating these injuries is to facilitate axon regrowth by enhancing the microenvironment for nerve regeneration. Tissue engineering, which employs stem cells to create transplantable components, is an emerging technique for repairing nerve lesions. The use of conduits in tissue engineering is crucial as they serve as a scaffold for cell attachment, migration, and differentiation during tissue formation. The 3D printing technique is a novel method for constructing scaffolding. This study aims to investigate nerve regeneration and functional improvement using Schwann cells and a 3D-printed polyacrylonitrile conduit in a rat sciatic nerve injury model.
Materials and Methods: A polyacrylonitrile conduit was fabricated using a 3D printer for this study. Wharton's jelly mesenchymal stem cells (WJMSCs) were extracted from human umbilical cords and characterized by positive markers CD150, CD90, CD73, and negative markers CD35, CD45 using flow cytometry. Their differentiation potential into osteoblast and adipocyte cells was also confirmed. The WJMSCs were then cultured in the conduit and differentiated into Schwann cells (SCs). Fifteen male adult Wistar rats weighing 200-250 g were divided into three groups: 1. control group without treatment, 2. conduit group, and 3. conduit/SCs group. The conduits containing SCs were transplanted into the sciatic nerve lesion of the rats. After eight weeks, histological examinations were used to assess nerve regeneration. The quality of the scaffold was evaluated using a scanning electron microscope (SEM), and the quality of SCs on the conduit was assessed using acridine orange and DAPI staining. The differentiation of cells was assessed using qRT-PCR and immunocytochemistry.
Results: The results demonstrated that WJMSCs were successfully differentiated into SCs under the differential condition used in this study. Immunocytochemistry and qRT-PCR analysis confirmed the expression of P75 and S100 markers. The 3D-printed PAN polymer was a suitable method for preparing the conduit, and the SCs exhibited high attachment and survival rates on the conduit. SEM and acridine orange and DAPI staining showed the establishment and survival of a high percentage of SCs on the conduit. In the histological study, the treated groups showed higher numbers of axons, epineurium, Schwann cells, and fascicle formation compared to the control group.
Conclusion: This study indicates that tissue engineering combined with conduits can have a positive effect on the treatment of peripheral nerve injuries. The use of 3D printing technology in the preparation of conduits for SCs has the potential to be a promising approach in nerve regeneration therapy.
Keywords: Conduit, Mesenchymal Stem Cells, Neural Tissue Engineering, Sciatic Nerve Injury

Os-2: Surface Energy Investigation of Polyurethane/ Poly(amidoamine) Dendrimer Composite for Cardiovascular Applications

Elahi O[1*], Solouk A[1], Akbari S[2]

1. Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
2. Department of Textile Engineering, Amirkabir University of Technology, Tehran, Iran
Email: atefeh.solouk@gmail.com

Objective: Polyurethanes (PU) are promising materials for biomedical applications, especially in the cardiovascular field. Incorporating poly(amidoamine) dendrimers (D) has shown improvements in antibacterial activity and conductivity, but their impact on surface energy remains largely unexplored. Surface energy plays a crucial role in biomaterials' cell-supporting behaviours such as cell adhesion and proliferation, making it an important factor to consider.
Materials and Methods: In this study, we investigated the polar and dispersive surface energy of PU/D composite casted films using the sessile drop method and measuring contact angles with water and diiodomethane. The Owens-Wendt model was employed to determine the surface energy of the films.
Results: The polar surface energies of the PU film, along with films containing 2 wt% dendrimer (D2), 4 wt% dendrimer (D4), and 8 wt% dendrimer (D8), were found to be about 5.98 , 8.20, 8.71, and 12.16 mN/m, respectively. Notably, all changes in surface energy were observed in the polar component, while the dispersive component remained consistent across all samples. The overall surface energies of the PU, D2, D4, and D8 samples were measured as about 43.37, 44.64, 47.50, and 51.72 mN/m, respectively. The observed increase in surface energy can be attributed to the presence of surface amine groups on the PAMAM dendrimer.
Conclusion: Higher surface energy is known to promote better cell adhesion and proliferation. These findings provide valuable insights into the influence of polyurethane modification with PAMAM dendrimers on surface energy, which can aid biomaterial design.
Keywords: Biomedical Applications, Cardiovascular, PAMAM Dendrimers, Polyurethanes, Surface Energy

Os-3: CML-derived hematopoietic stem cells treated by imatinib loaded in EV from BM-MSCs

Esmaeilirad A[1*], Hashemi ZS[2], Hosseinpour Feizi MA[1], Safaralizadeh R[1], Sarrami Forooshani R[2]

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tabriz University, Tabriz, Iran
2. Department of ATMP, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
Email: z.hashemi@acecr.ac.ir

Objective: Bone marrow mesenchymal stem cells (BM-MSCs) are the most common MSC source. These cells can attract to the tumor microenvironment by following chemokines, cytokines, and growth factors secreted by tumor cells. Tumoral attracting

properties of MSCs-derived extracellular vesicles (EVs) are considered as their parent cells. So, drug loading into EVs as the carrier can be used for targeted. Here, we investigated the delivery of Imatinib to the CML-derived hematopoietic stem cell (HSCs) by EVs.
Materials and Methods: Human mononuclear cells (MNCs) were separated from the donor's healthy BM by Ficoll solution. After two weeks, adherent BM-MSCs were grown to 70%–80% confluency and incubated for 48h in serum-free media. EVs were isolated and characterized by DLS and TEM from expansion media. Imatinib was loaded via the ultrasonication method to obtain Imatinib-EVs formulation. HSCs were isolated from BM of CML patient by the MACS isolation kit. The cytotoxic effects were investigated by MTT and Real-Time PCR.
Results: The spindle shape morphology of BM-MSCs was observed and confirmed by CD marker expression (flow cytometry analysis for CD105 and CD45) and their multipotent differentiation capacity into osteocytes. Particle size of EVs was analyzed using DLS as 187nm. The EVs morphology was confirmed by TEM. The efficacy of Imatinib loading was calculated as 52% (encapsulated/ total). All viability assays confirmed the more inhibitory effects (78%) of Imatinib-EVs compared to the free Imatinib.
Conclusion: EVs-based therapy as the cell-free therapy along with the cargo of chemotherapy drugs showed a potent inhibitory effect on proliferation and induced apoptosis on human CML-derived HSCs. The result displayed that Imatinib-EVs selectively reduced tumor cell viability. All data confirm that they can be used as drug carriers with selective toxicity to the normal cells to reduce the side effects of chemotherapeutic drugs.
Keywords: CML, Extracellular Vesicles, Hematopoietic Stem Cells, Mesenchymal Stem Cells, Imatinib

Os-4: 3D Culture of K562 on Bone Matrix Scaffold Treated by NK-Derived Exosome

Esmaeilirad A[1*], Hashemi ZS[2], Sarrami Forooshani R[2]

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tabriz University, Tabriz, Iran
2. ATMP Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
Email: z.hashemi@acecr.ac.ir

Objective: Tissue engineering is a multidisciplinary field with new and exciting techniques. In the meantime, scaffolds are proposed to create a similar environment in *ex vivo* conditions and to increase the volume of the culture medium. 3D culture using a bone matrix scaffold to mimic the bone marrow microenvironment might facilitate the study of K562 (representative of Chronic Myelogenous Leukemia (CML) cells in bone marrow) in its native 3D niche. Anti-tumoral features of exosomes released by natural killer cells (NK-Exos) are considered their parent cells capable of fighting virally infected and tumorous cells. Here, the treatment by NK-Exos in a 3D model was assessed.
Materials and Methods: Bone matrix scaffold was prepared from the spongy part of the bovine's bone marrow. After various treatments with chemical materials and the use of gamma rays to eliminate the risk of contamination, the structure of the bone matrix scaffold was obtained in the form of a porous matrix. So, the scaffolds were prepared in the form of slices to be placed on the floor of the ideal cell culture platform for K562. NK cells were obtained from peripheral blood mononuclear cells (PBMC) and NK-Exos were isolated from NK cell expansion media. The cytotoxic effect of NK-Exos on 3D culture of K562 in bone matrix scaffold was monitored by MTT, Real-Time PCR for P53 and VEGF-A.
Results: Our results showed that the bone matrix scaffold supported the best seeding efficiency and leukemic growth. The MTT assay indicated that NK-Exos selectively reduced tumor cell viability up to two times compared to the 2D culture group. The results confirmed the exosome delivery system in an *ex vivo* environment, which mimics *in vivo* conditions, has enabled better.
Conclusion: In order to improve the efficiency of cell and tissue culture in tissue engineering, it is necessary to create the three-dimensional conditions of the body (*in vivo*) in the external state (*ex vivo*). The scaffold is based on materials in the extracellular matrix that have been subjected to various treatments. Therefore, the obtained porous bone scaffold with its geometrical structure and 3D topography can imitate the 3D microenvironment of the bone marrow to some extent if it is coated with K562 cells. With this simulation, all kinds of new drug treatments and their results can be done easily. Here, NK-Exos showed a potent inhibitory effect on proliferation and induced apoptosis on CML-modeling bone marrow.
Keywords: Bone Matrix Scaffold, CML, 3D Culture, Exosome, Natural Killer Cells

Os-5: KDM6A Over-Expression Attenuated Cancerous Phenotype in Hepatocellular Carcinoma Cells

Hashemi M[1, 3*], Vosough M[2], Hesaraki M[1], Torabi Sh[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 Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Molecular Cell Biology-Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
Email: masvos@royaninstitute.org

Objective: Hepatocellular carcinoma (HCC), is the most common hepatic cancer and the third leading cause of death for those with chronic liver disease. Even with established treatment approaches, recurrence rates of this cancer are very high in patients after surgery. Therefore, researchers are seeking new therapeutic approaches such as targeted molecular therapies to prevent disease replases. KDM6A is part of the histone demethylase family which plays a crucial role in the suppression of tumors in gastrointestinal cancers.,Mutations of this gene results in increased proliferation, migration, invasion, and metastasis capacity of cancer cells. In this regard, pharmacologically targeting KDM6A could be a novel therapeutic modality. The purpose of this study is to induce KDM6A expression in hepatocellular carcinoma cells in order to reduce cancerous characteristics.
Materials and Methods: A lentiviral vector-based vector was used to induce KDM6A overexpression in Huh-7 human hepatic cells. Then, the effect of KDM6A over-expression on the cancerous phenotype of cells was assessed by examining their proliferation, colony formation, and viability of cells.
Results: KDM6A overexpression altered the morphology, proliferation, viability, cell cycle pattern, and colony formation ca-

pacity, in HCC cells.
Conclusion: The induction of KDM6A activity could be used as a potential non-invasive therapeutic strategy for preventing cancer metastasis and recurrence.
Keywords: Cancerous Phenotype, Hepatocellular Carcinoma, Lentivirus Vector, KDM6A

Os-6: Extracellular Matrix Preconditioning Improves Retinal Pigment Epithelium Integration after Transplantation

Mazinani F[1*], Kazemi Ashtiani M[2], Satarian L[1], Baharvand H[1, 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 Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: l.satarian@royan-rc.ac.ir

Objective: Human pluripotent stem cell-derived retinal pigmented epithelium (hPSC-RPE) transplantation has become an attractive option for retinal degenerative diseases. However, numerous pathological changes in Bruch's membrane (BrM), make extracellular matrix (ECM) proteins out of the cell's reach, resulting in poor integration of transplanted cells in diseased eyes. *In vitro* experiments have revealed that conditioned medium (CM) derived from endothelial cells (EC) significantly increased RPE survival on AMD BrM explants due to increase in ECM deposition. These finding lead us to hypothesize that whether hESC-RPE preconditioning with RPE-CM and/or RPE-ECM, could increase ECM deposition and integration after transplantation in animal models.
Materials and Methods: To figure out the effects of RPE secreted factors on hPSC-RPE proliferation and monolayer formation, 5×10^4 cells/cm^2 were cultured in one of three conditions: 1. control; 2. 50% RPE-CM; and 3. 1 µg/ml RPE-ECM. At day 9, all the groups were collected and stained in primary antibodies against Ki-67 and ZO1. To evaluate the effect of preconditioning on RPE integration, after 21 days the GFP+ RPE cells were digested and directly injected to the subretinal space of C57Bl/6 mice model of retinal degeneration (n = 24). After 7 days, the eyes were examined for green fluorescent area (GFA%) related to transplanted cells. To assess spatial visual acuity after hPSC-RPE transplantation, 1×10^5 cells were injected to the subretinal space of RCS rats (n = 70) and the visual acuity was measured at 30 to 90 days post transplantation using OptoDrum system.
Results: Our results showed that RPE-ECM supplementation had no negative effects on cell viability and increased hESC-RPE growth slightly. Proliferation of hPSC-RPE supplemented with ECM was associated with improved hexagonal phenotype of the monolayer as evidenced by immunoflurecense staining of tight junction protein ZO1.Furtheremore, ECM treated RPE showed a significant more engraftment than the control group in mice as evidenced by median GFA evaluation. Interestingly the increased engraftment translated into improved spatial visual acuity (2.25 fold) in RCS rats. Also, *in vivo* immunofluorescence assay indicated that preconditioned hPSC-RPE not only remained in a non-proliferative state but also supported cone photoreceptor survival.
Conclusion: Our results suggest that preconditioning the RPE cells may increase ECM deposition and integration after transplantation, providing a potential solution for improving the viability of transplanted cells in AMD patients
Keywords: Extracellular Matrix, Integration, Preconditioning, Retinal Pigmented Epithelium

Os-7: Osteogenic Activity on Naoh-Etched 3d-Printed Poly-E-Caprolactone Scaffolds in Perfusion or Spinner Flask Bioreactors

Seddiqi H[1*], Abbasi-Ravasjani S[1], Saatchi A[1, 2, 3], Amoabediny Gh[2, 3], Zandieh-Doulabi B[1], Jin J[1], Klein-Nulend J[1]

1. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, Netherlands
2. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
3. Department of Biomedical Engineering, Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
Email: j.kleinnulend@acta.nl

Objective: Spinner flask and perfusion bioreactors, and cell-seeded 3D-printed scaffolds are used in bone tissue engineering to stimulate cells and produce bone for implantation into patients. Wall shear stress (WSS) induced by spinner flask and perfusion bioreactors crucially affects cell function and might differentially affect osteogenic activity. We fabricated surface-modified 3D-printed poly-ε-caprolactone (PCL) scaffolds, and static, spinner flask, and perfusion bioreactors to determine WSS and osteogenic responsiveness of pre-osteoblasts on the scaffolds in the bioreactors using finite element (FE)-modeling and experiments.
Materials and Methods: FE-modeling was used to determine WSS inside 3D-printed PCL scaffolds within spinner flask and perfusion bioreactors. MC3T3-E1 pre-osteoblasts were seeded on NaOH surface-modified 3D-printed PCL scaffolds, and cultured in static, spinner flask, and perfusion bioreactors up to 7 days. The scaffolds' physicochemical properties and pre-osteoblast function were assessed experimentally.
Results: FE-modeling showed that the bioreactors locally affected WSS distribution and magnitude. WSS distribution was more homogeneous in perfusion than in spinner flask bioreactors. Average WSS ranged from 0–6.5 mPa (spinner flask bioreactors) and from 0–4.1 mPa (perfusion bioreactors). Surface-modification resulted in a honeycomb-like patterned surface, and increased surface roughness (1.6-fold), but decreased water contact angle (0.3-fold). Both bioreactors increased cell spreading, proliferation, and distribution. Perfusion, but not spinner flask bioreactors more strongly enhanced collagen (2.2-fold) and calcium deposition (2.1-fold) compared to static bioreactors, due to uniform WSS-induced mechanical stimulation of cells revealed by FE-modeling.
Conclusion: Our findings indicate the importance of using accurate FE models to estimate WSS and determine experimental conditions for designing cell-seeded 3D-printed scaffolds in bioreactor systems.
Keywords: Bioreactor, Bone Tissue Engineering, Finite Element Modeling, Osteoblasts, 3D-Printed Scaffold

Os-8: Potential Antiaging Activity of Secretome Gel of Human Wharton's Jelly Mesenchymal Stem Cells in UV-In-

duced Mice Models

Widowati W[1*], Faried A[2, 3], Rahmat D[4], Widya Kusuma HS[5], Mia Dewi N S[5], Gondokesumo ME[6], Rizal Rizal[5, 7]

1. Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
2. Department of Neurosurgery, Faculty of Medicine, Padjadjaran University, Dr. Hasan Sadikin Hospital, Bandung, Indonesia.
3. Oncology and Stem Cell Working Group, Faculty of Medicine, Padjadjaran University, Dr. Hasan Sadikin Hospital, Bandung, Indonesia
4. Faculty of Pharmacy, Pancasila University, South Jakarta, Indonesia
5. Biomolecular and Biomedicine Research Center, Aretha Medika Utama, Bandung, Indonesia
6. Faculty of Pharmacy, University of Surabaya, Surabaya, Indonesia
7. Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, Indonesia.
Email: wahyu_w60@yahoo.com

Objective: Skin aging is a degenerative process that can be induced by ultraviolet (UV) irradiation. UV radiation can produce oxidative stress which causes premature aging. Research objective: This study aims to examine the antiaging potential of secretome gel from human Wharton Jelly Mesenchymal Stem Cells (hWJ-MSCs) in a UV-induced mice model.
Materials and Methods: The secretome was obtained from hWJ-MSCs and made in gels form. Male mice were radiated by UV for 15 minutes twice daily for 14 days. The gels were topically applied to the dorsal mice's skin. Two treatments of secretome gel; secretome 1 is applied 1 time and secretome 2 is applied 2 times daily after UV radiation. The Tumor Growth Factor β (TGF-β), Interleukin (IL) 10, and IL-18 gene expression were determined using real-time polymerase chain reaction (RT-PCR). Hematoxylin Eosin staining was used to observe the histopathology and collagen skin tissue structure. An immunohistochemistry assay was used to analyze the protein expression of collagen (COL)-4A1, Matrix Metalloprotease (MMP) 2, MMP 13, and p53.
Results: UV induction caused loss of collagen, increasing inflammation and high expression of aging mediators. Secretome gel increased the gene expression of TGF-β, IL-10 and decreased IL-18 gene expression. Histopathological tests showed that gel secretome increased collagen density and lowered inflammation and repair cell damage in skin tissue. Immunohistochemistry test showed that gel secretome decreased MMP-2, MMP-13, and p53 protein expression, in contrast, increased COL4A1.
Conclusion: Secretome gel of hWJ-MSCs showed antiaging activities that potential for preventing skin aging.
Keywords: Inflammation, Mesenchymal Stem Cells, Secretome, Skin Aging, Wharton jelly

Poster Presentations

Ps-1: Arginine-Glycine-Aspartate Immobilization on Carboxyl Surface-Functionalized Electrospun Poly-E-Caprolactone Scaffolds Promotes Endothelialization and Anti-Thrombotic Activity in a Perfusion Bioreactor

Abbasi-Ravasjani S[1*], Seddiqi H, Safari-Hajataghaei MR[2], Amoabediny Gh[2, 3], Jin J[1], Hajiani F[4], Zandieh-Doulabi B[1], Klein-Nulend J[1]

1. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA)-University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, Netherlands
2. Department of Biomedical Engineering, Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
4. Department of Textile and Clothing Design, Faculty of Applied Arts, University of Art, Tehran, Iran
Email: j.kleinnulend@acta.nl

Objective: Rapid endothelialization, cell stability, and prevention of thrombus formation by nitric oxide (NO) production, at the lumen of vascular electrospun poly-ϵ-caprolactone (PCL) scaffolds under flow is a challenge. Surface-functionalization of PCL scaffolds with negatively-charged carboxyl (COOH), or positively-charged amine (NH2) groups followed by immobilization of arginine-glycine-aspartate (RGD) onto a scaffold surface affects endothelialization and cell stability. However, whether RGD-immobilization on COOH surface-functionalized electrospun PCL scaffolds is more effective than on NH2 surface-functionalized scaffolds under blood flow is unknown. We aimed to test whether RGD-immobilization on COOH or NH2 surface-functionalized electrospun PCL scaffolds affects endothelialization, endothelial cell stability, and NO production in a perfusion bioreactor mimicking blood flow.
Materials and Methods: Endothelial cells were seeded on electrospun PCL scaffolds surface-functionalized by COOH or NH2 groups followed by RGD immobilization, and cultured up to 8 days in a static or perfusion bioreactor. Scaffolds' physicochemical properties, endothelialization, cell stability, and NO production were investigated.
Results: COOH and NH2 surface-functionalization followed by RGD immobilization decreased fiber diameter (0.4-0.5-fold) and water contact angle (0.3-0.7-fold), but increased pore size (1.7-2.1-fold) and porosity (1.2-fold). COOH and NH2 surface-functionalization followed by RGD immobilization increased cell proliferation (2.2-5.6-fold) and collagen deposition (1.4-1.7-fold) after 8 days in a perfusion bioreactor and increased cellular NO production (1.2-4.2-fold) after 30 minute in a perfusion bioreactor. Cells were more stable on COOH surface-functionalized and RGD immobilized scaffolds after 1 hours in a perfusion bioreactor.
Conclusion: Maximum endothelialization, cell stability, and NO production was observed on carboxyl surface-functionalized electrospun PCL scaffolds followed by RGD immobilization, which might be an excellent candidate for long-term application of endothelial cells to prevent thrombus formation at the lumen of artificial vascular grafts under blood flow.
Keywords: Amine,Carboxyl, Endothelialization,Polycaprolactone, RGD

Ps-2: The Effect of Hydrogel Derived from Decellularized Mouse Renal Papilla on Mouse Renal Tubular Epithelial Cells

Abdi Sh[1, 2*], Saniefar H[1, 2], Erfanian S[2], Kazemi-Ashtiani M[2, 3], Rajabi S[2, 3], Moghadasali R[1, 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 Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
3. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: Rezamoghadasali@Royaninstitute.org

Objective: The extracellular matrix (ECM) plays a crucial role in maintaining tissue homeostasis and regulates cell behavior. The renal papilla is recognized for being a niche for residing stem cells. The unique ECM composition in this area is the key element in preserving developmental potencies. Tubular epithelial cells (TECs) are one of the populations participating in kidney regeneration, residing in the cortex and medulla. They undergo epithelial to mesenchymal transition (EMT) after isolation and lose their original characteristics. In this study, it is expected that by mimicking the natural conditions in the lab using papilla decellularized extracellular matrix (dECM) in hydrogel form, the EMT occurrence can be prevented in order to come up with a safe and efficient approach to omit the complications in using TECs for further applications.
Materials and Methods: Papilla, cortex, and medulla of mouse kidney were decellularized using SDS 0.5% for 24 hours and washed in deionized water for 12 hours, then characterized using DNA quantification, Sircol, and Blyscan assays, and histology staining (H&E, Alcian Blue, Sirius Red, and DAPI). Hydrogel was obtained by digesting the lyophilized dECM with pepsin in an acidic pH= 1-2. TECs were isolated from FVB/N mouse kidney cortex and cultured in RPMI advance, with 15% FBS. TECs were encapsulated in obtained hydrogels. Viability and metabolic activity were evaluated using MTS and Live/Dead assays on day 3 and 7, and DNA quantification was used for proliferation assessment. Ultimately, Cdh1 and Tjp1 as epithelial markers, and Vim, Mmp9 as mesenchymal markers expression was evaluated using qRT- PCR.
Results: Based on results obtained by DNA quantification, ECM characterization and histological evaluations, Collagen and GAG content were preserved (P value<0.512) while DNA content was excluded from both tissues (P value<0.0001). MTS, Live/Dead and DNA quantification assays determined TECs was more proliferative in cortex and medulla hydrogel, and gene expression indicated EMT occurring in this hydrogel. TECs cultured in papilla hydrogel showed more metabolic activity while proliferation was not significant. Also, gene expression indicated epithelial morphology in papilla hydrogel.
Conclusion: TECs were more proliferative in the cortex and medulla hydrogels which is composed of the ECM derived from their natural niche and as a result of that, EMT was promoted in this hydrogel, while papilla hydrogel was better in maintaining epithelial characteristics and less proliferation was seen.
Keywords: Extracellular Matrix, Decellularization, Renal Pa-

pilla, Tubular Epithelial Cells

Ps-3: Anticancer Evaluation of Metformin Drug and Silver Nanoparticles Co-Encapsulated Niosomes Against Lung Cancer Cell Line

Aghapour N*, Johari B, Musazadeh N

Department of Medical Biotechnology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
Email: dr.johari@zums.ac.ir

Objective: The aim of the present investigation was to develop niosomes containing metformin drug and silver (Ag) nanoparticles as radiosensitizer agents. Also, the combinational effect of metformin (Met) drug and Ag along with X-irradiation exposure in both free and Met-Ag encapsulated Niosome forms on growth inhibition potential and induction of apoptosis in lung cancer (MDA-MB-231) cell line were exploited.
Materials and Methods: Niosomes were prepared by thin-film hydration method and conjugated with Ag nanoparticles. Their physicochemical properties were determined by various techniques (DLS, FT-IR, FE-SEM, and hemolysis assays). Cellular uptake, cell apoptosis, wound healing, and MTT assays were conducted to ascertain niosomes feasibility for cancer therapy.
Results: The combination of Met and Ag in niosomal formulation showed inhibited cell growth and induced apoptosis than their combination in free form under X-ray conditions.
Conclusion: The nanocarrier-based approach was effective for the co-delivery of Met and Ag against cancer cells *in vitro*.
Keywords: Apoptosis, Combination Therapy, Lung Cancer, Niosomes, Metformin

Ps-4: Oxidative Stress Induced by Di-(2-ethylhexyl) Phthalate Was Compensated by Catechin Hydrate to Improve Osteogenic differentiation of Bone Marrow Mesenchymal Stem Cells

Ahmadi Z*, Abnoosi MH

Department of Biochemistry, Arak University, Arak, Iran
Email: mzargolahmadii1996@gmail.com

Objective: Di-(2-ethylhexyl) phthalate (DEHP) is an economical and commonly used plasticizers, which humans are exposed through polyvinyl chloride products. It is known that DEHP hinders osteogenic differentiation of Bone marrow mesenchymal stem cells (BMSCs) and as result reduces the production of bone matrix via its induction of oxidative stress. Since catechin hydrate (CH) was introduce to inhibit oxidative stress, it is used to ameliorate osteogenic inhibition of DEHP.
Materials and Methods: BMSCs were extracted from Wistar rats and treated with DEHP (100 µM) and CH (0.25 µM) individually and in combination for 20 days. Then matrix production, total protein content, alkaline-phosphatase activity, MDA level, total antioxidant capacity, CAT and SOD activity and osteogenic related gene expression were analyzed.
Results: Data showed, treatment with DEHP inhibit bone matrix production, whereas CH compensates its effect. Also, a significant increase in the expression of BMP2/ RUNX2 pathway genes were observed, which increased ALP activity. CH compensated the oxidative stress caused by DEHP via improving the activity of antioxidant enzymes and reducing the amount of MDA.
Conclusion: CH was able to compensate the toxic effect of DEHP, and improved osteogenic differentiation of BMSCs by elevation of BMP/RUNX2 pathway genes expression.
Keywords: BMSCs, Catechin Hydrate, DEHP, Osteogenic Differentiation, Oxidative Stress,

Ps-5: To Examine The Effect of Stress Caused by Chronic Noise Exposure on Cistauosis Incidence in Rats

Ahmadzadeh Naqadeh Z[1*], Emami M[2], Shahpasand K[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 Biotechnology, College of Science, University of Tehran, Tehran, Iran
Email: shahpasand09@gmail.com

Objective: Chronic noise exposure as a stressor causes disturbances in the functioning of the central nervous system and the occurrence of behavioral and cognitive disorders such as Alzheimer's disease (AD). One of the most important pathological symptoms of AD is tauopathy caused by hyperphosphorylated tau protein, an important protein related to the neuron cytoskeleton. One of the key changes in tau protein in this process is the conversion of the threonine231-proline bond from trans to cis conformation due to the phosphorylation of threonine. this pathological form of the tau protein is called 'cistauosis', which, if prevented, can reduce neurological disorders such as Alzheimer's disease.
Materials and Methods: The study aims to investigate the effect of chronic noise stress on cognitive functions and the occurrence of cistauosis in male Wistar albino rats. Twenty rats were randomly divided into the noise-exposed group and the control group, and rats in the exposure group were exposed to 95 decibels of sound pressure level (dB SPL) white noise for 15 consecutive days (4 hours per day). we used behavioral (elevated plus maze and Y maze) and molecular tests (western blotting and immunofluorescence) to investigate the idea.
Results: Chronic noise stress led to a decrease in cognitive function as evidenced by increased anxiety-related behaviors and poor memory in elevated plus maze and Y maze, respectively. The effects of noise on the occurrence of cistauosis in different parts of the brain, such as the cerebral cortex, hippocampus, and brainstem which were surgically separated, were investigated by western blotting and immunofluorescence against the cis form of tau protein. The results showed that chronic noise exposure significantly (P value < 0.05) causes cistauosis in all the different examined parts of the brain, especially the cortex.
Conclusion: These observations show that chronic noise stress by inducing mental stress leads to cistauosis and as a result, provides the basis for the development of neurological disorders and the occurrence of diseases such as Alzheimer's.
Keywords: Alzheimer's Disease, Chronic Noise Exposure, Cistauosis, Cognitive Disorders, Taupathy

Ps-6: Characterization of Extracellular Vesicles (EVs) Isolated from Human Adipose Mesenchymal Stem Cells (AD MSC) Conditioned Medium as Potential Bone Tissue Regeneration Inducer

Antarianto RD[1, 2*], Kousar I[2, 3], Hilmy F[4], Makabori J, Chrysant F[4], Margaret AL[5], Andini MA[6]

1. Department of Histology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
2. Stem cell and Tissue Engineering Research Cluster, IMERI FKUI, Jakarta, Indonesia
3. Master Program in Biomedical Science, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
4. Orthopaedic and Trauma Surgery, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
5. Department of Biomedical Science, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
6. Department of Biology, Faculty of Mathematics and Natural Science Universitas Indonesia, Depok, Indonesia
Email: radiana.dhewayani@ui.ac.id

Objective: Critical size bone defect remains a significant challenge in orthopedic and trauma patients. Failure of bone graft integration, lack of proper bone regeneration, inadequate vascularization, imbalance between soft callus and hard bone formation are the unresolved problems. Aim of this study is to investigate the potential of EVs AD MSC as inducer for bone regeneration.
Materials and Methods: This study has been conducted since February 2022 and on going at Department of Histology FKUI and SCTE IMERI FKUI. EVs AD MSC was isolated from AD MSC conditioned medium by series of different centrifugations to remove cell debris, apoptotic bodies, large EVs and ended with ultracentrifugation at IMERI. Sterility testing against 23 specific bacterial and viral pathogens was conducted at Microbiology lab FKUI (LMK FKUI). Particle size and zeta potential measurement was performed using Horiba SZ100 at ILRC UI Depok. Enrichment of EVs AD MSCs was done with Miltenyi exosome isolation kit (CD63) and phenotyped for CD81 antibody at SCTE IMERI. Proteomics analysis was done using LC MS/MS at RS POLRI Lab. miR profiling was outsourced at NANOSTRING lab in Singapore.
Results: Sterility testing showed negative detection of specific pathogens from EVs AD MSC. Mean nanoparticle size of EVs AD MSC ranges between 88.4 nm to 123.9nm (n=3) with monodisperse, homogeneous distribution. Mean zeta potential showed electronegative charge of EVs AD MSCs (-10,2 mV, -4.3 mV and -1.2mV). Enrichment of exosome from EVs AD MSC showed double positive CD63 and CD81 in majority of the nanoparticles. LC MS/MS result of EVs AD MSC revealed 9 parent ions and 2 daughter ions with different abundance. Further identification with PubChem revealed the abundance of the following chemical compound: 4-ethyl-1H-pyrazol-5-amine; 2-formylbutanenitrile; bis(2-methoxyethyl)azanium; 2-amino-1-(5-cyanotetrazol-2-yl)oxyguanidine and 4-amino-1,3-dimethyl-2,6-dioxopyrimidine-5-carbaldehyde. miR profiling of EVs AD MSC revealed over 700 human miR present from the isolated EVs AD MSC. Several miR detected in EVs AD MSC that has been reported to induce bone regeneration are miR-129-5p, miR-140-3p, miR-142a-3p, miR-142-5p, miR-190a-5p, miR-199b-5p, miR-217, miR-335-5p.
Conclusion: EVs AD MSC isolated at SCTE IMERI are sterile agains specific pathogens, fullfilled ISEV minimal criteria as exosome (size less than 150nm and expresses CD63+/CD81+), able for endocytosis (electronegative charge) and contained several miRs to induce bone regeneration.
Keywords: AD MSC, Bone Regeneration, Exosome, EVs, miR

Ps-7: Fabrication and Characterization of Nitric Oxide-Loaded Microparticles for Wound Healing Applications

Bagheri Azizabad Z*, shabani I

Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: shabani@aut.ac.ir

Objective: The process of wound healing involves inflammation, cell proliferation, matrix deposition, and tissue remodeling, all highly coordinated. Deficiencies in any of the steps may result in chronic wounds. Bacterial infections may also delay wound healing. Endogenous free radicals such as nitric oxide (NO) possess powerful antimicrobial, vasodilatory, smooth muscle relaxant, and growth factor stimulation properties. NO is unstable when exposed to biological environments, which limits its wide biomedical applications. The aim of this study is to develop site-specific controlled release systems for NO and to enhance its stability for use in wound dressings.
Materials and Methods: GSNO powder was synthesized via a reaction between reduced glutathione (GSH) and acidified nitrite. Briefly, 3.1 mmol GSH dissolved in 5.0 mL of 0.626 M HCl cooled to 4°C under nitrogen flow. After the filtration and washing, the pink powder was dried under freeze-drying. Utilizing a water-in-oil-in-water double emulsion solvent evaporation method, the NO donor S-nitroso glutathione (GSNO) was encapsulated in poly (L-lactic acid) (PLA) microparticles. The microparticles were characterized using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). NO-releasing was investigated by Griess assay.
Results: The spherical morphology of the microparticles was examined by SEM. The chemical structure was confirmed by FTIR. Sustained NO delivery was observed over a period of 7 days following NO release.
Conclusion: The results show that the prepared microparticle-based system with continuous release of nitric oxide can be useful for wound dressing applications.
Keywords: Microparticles, Nitric Oxide, Wound Dressing

Ps-8: Investigation of Autograft and Xenograft Platelet-Rich Plasma in The Treatment of Multiple Sclerosis Induced by Cuprizone

Balasi N[1*], Nazm Bojnordi M[1], Ghasemi Hamidabadi H[1], Nouri M[2]

1. Department of Anatomical Sciences, Mazandaran University of Medical Sciences, Mazandaran, Iran
2. Royan Stem Cell Technology Company, Ideal Gene and Cell Company, Tehran, Iran
Email: nesa.balasi68@yahoo.com

Objective: Multiple sclerosis(MS) is one of the most common neurodegenerative diseases in which the myelin sheath that forms the axon in neurons of the central nervous system is destroyed. Platelet-rich plasma(PRP)is called a growth factor concentrate,and PRP-derived Exosomes(PRP-Exos)have the same effect as their parent material.The aim of this research is to compare the effect of platelet-rich plasma between human and rat species in myelinrepair caused by cuprizone in the MS model.
Materials and Methods: The present study, the demyelination model was induced using an oral regimen of cuprizone 0.2 for 6weeks. After the allograft group of autologous PRP, blood was taken from healthy mice through heart punctures. In the xenograft group of blood from Royan Stem cells company,where

human PRP was used. extension demyelination and Remyelination investigated were evaluated Luxol Fast Blue (LFB).also,in order to Movement changes of BBB and Foot Print was investigated.
Results: In the autograft and allograft groups,myelin repair increased significantly and was confirmed by the (LFB)method Functional recovery was confirmed via behavioral tests in BBB and Foot Print groups.
Conclusion: With local administration of PRP, they are promising treatments for motor and sensory indicators,as both show neuroregenerative and neuroprotective properties.
Keywords: Autograft PRP, Cuprizone, Multiple Sclerosis, Myelin, Xenograft PRP

Ps-9: The Effect of Platelet-Rich Plasma Injection on Increasing Myelin Repair Capacity in Cuprizone-Induced Multiple Sclerosis Model Rats

Balasi N[1*], Nazm Bojnordi M[1], Ghasemi Hamidabadi H[1], Nouri M[2]

1. Department of Anatomical Sciences, Mazandaran University of Medical Sciences, Mazandaran, Iran
2. Royan Stem Cell Technology Company, Ideal Gene and Cell Company, Tehran, Iran
Email: nesa.balasi68@yahoo.com

Objective: Multiple sclerosis (MS)is one of the most common neurodegenerative diseases in which the myelin sheath that forms the axon in neurons of the central nervous system is destroyed. Platelet-rich plasma (PRP)is a growth factor concentrate derived from PRP. The purpose of this research is the effect of PRP injection in the MS model induced by cuprizone in order to evaluate remyelination and behavioral function in male rats.
Materials and Methods: The demyelination model was induced using an oral regimen of cuprizone 0.2 for 6 weeks. After that, blood was obtained from Royan Stem cells company y and after centrifugation, PRP was injected into mice. extension demyelination and Remyelination investigated were evaluated Luxel Fast Blue. Also, in order to movement changes of BBB and Foot Print was investigated.
Results: In the allograft groups, myelin repair increased significantly and was confirmed by the Luxel Fast Blue method Functional recovery was confirmed via behavioral tests in BBB and Foot Print groups.
Conclusion: By topical administration of PRP, they are promising treatments for motor and sensory indicators, as they both exhibit neuroregenerative and neuroprotective properties.
Keywords: Cuprizone, Multiple Sclerosis, Myelin, PRP

Ps-10: Effect of Human Placental ECM-Derived 3D Printed Scaffold on Bone Defect Healing *In vivo*

Bashiri Z[1*], Gholipour Malekabadi M[2]

1. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: zbashiri88@gmail.com

Objective: Although surgery and autograft are the standard method for repairing large defects in bones, this technique has limitations including limited resources and donor site complications. Extracellular matrix (ECM)-based printed scaffolds can be used to heal large bone defects.
Materials and Methods: In this study, human placenta tissue was decellularized using SDS-Triton X-100 and then solubilized with urea. To create a native 3D scaffold resembling bone, several concentrations of ECM solution (0, 1.5, 3, and 5%w/v) were combined with 8% Alginate (Alg)/12% Silk Fibroin (SF) for printing. Then their structural, mechanical and biological properties were characterized. Finally, the best 3D printed scaffold (5% w/v) was investigated as a bone graft for a skull bone defect in a rat model.
Results: This study demonstrated the decellularization of placental tissue fragments efficiently led to the removal of cell debris. Also, by increasing the ECM concentration, an important improvement in the structural, mechanical and biological properties of the printed scaffolds was observed. The results of histology studies and Real time PCR analysis confirmed better improvement of bone formation in defects treated with 5% ECM-SF/Alg in 4 weeks compared to the group without ECM. Bone regeneration was clearly improved 8 weeks after implantation.
Conclusion: Overall, these findings proved that ECM printed scaffolds, by simulating the native bone microenvironment, have the potential to construct biomimetic grafts for the reconstruction of large bone defects.
Keywords: Human Placenta, Extracellular Matrix, Alginate/Silk Fibroin, Bone Defect Healing

Ps-11: 3D-Printed Placental-Derived Bioinks for Skin Tissue Regeneration with Improved Angiogenesis and Wound Healing Properties

Bashiri Z[1*], Gholipour Malekabadi M[2]

1. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: zbashiri88@gmail.com

Objective: Extracellular matrix (ECM)-based bioinks has attracted much attention in recent years for 3D printing of native-like tissue constructs. Due to organ unavailability, placental ECM can be an alternative source for the construction of 3D scaffolds for vascular regeneration in the treatment of deep wounds.
Materials and Methods: In this study, we use different concentrations (1.5, 3 and 5% w/v) of ECM-derived from placenta, and sodium-alginate and gelatin to prepare an printable bioink biomimicking natural skin. The morphology, physical structure, mechanical behavior, biocompatibility and angiogenic property of the printed hydrogels are investigated. The optimized ECM (5%w/v) 3D printed scaffold is applied on full-thickness wounds created in a mouse model.
Results: Due to their unique native-like structure, the ECM-based scaffolds provide a non-cytotoxic microenvironment for cell adhesion, infiltration, angiogenesis, and proliferation, whereas they don't show any sign of immune response to the host. Notably, the biodegradation, swelling rate, mechanical property, cell adhesion and angiogenesis properties increase with the increase of ECM concentrations in the construct. The

ECM 3D printed scaffold implanted into deep wounds increases granulation tissue formation, angiogenesis, and re-epithelialization due to the presence of ECM components in the construct, when compared with printed scaffold with no ECM and no treatment wound.
Conclusion: Overall, our findings demonstrate that the 5% ECM 3D scaffold supports the best deep wound regeneration *in vivo*, produces a skin replacement with a cellular structure comparable to native skin.
Keywords: 3D Printed Scaffold, Extracellular Matrix, Placenta, Wound Healing

Ps-12: Detection of MicroRNA-128 Involved in The Pathogenesis of Acute Lymphoblastic Leukemia with Electrochemical Nanobiosensor based on Reduced Graphene Oxide and Gold Nanoparticles

Basir Haghighi N[1*], Mohammad Nejad J[1], Yazdian F[1], Shabani Shayeh J[2], Omidi M[2], Pourmadadi M[3]

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Iran
2. Protein Research Center, Shahid Beheshti University, Tehran, Iran
3. Department of Biotechnology, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
Email: niki.basir@gmail.com

Objective: Nanobiosensors can improve diagnostics through rapid, specific, and sensitive detection of biomarkers. Acute lymphoblastic leukemia (ALL) is a prevalent cancer in children. micoRNA-128 is one of the useful biomarkers not only for diagnosis of ALL, but also for discriminating ALL from acute lymphoblastic myeloid (AML).
Materials and Methods: In this study, a novel electrochemical nanobiosensor based on reduced graphene oxide (RGO) and gold nanoparticles (AuNPs) has been fabricated to detect miRNA-128. Hexacyanoferrate as a label-free material was used in the design of the nanobiosensor. Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV) and Electrochemical Impedance Spectroscopy (EIS) have been applied to characterize the nanobiosensor.
Results: The results show that the modified electrode has excellent selectivity and sensitivity to miR-128 with the limit of detection of 0.08761. FTIR, XRD and SEM analysis displayed the quality of the synthesized nanomaterials. The stability and diffusion control analyses were performed as well. Also, 5 new cases of ALL, 3 AML and 6 control serum samples were used for the measurement of the selectivity and sensitivity of the fabricated nanobiosensor.
Conclusion: Examination of real serum samples from ALL and AML patients and control cases confirmed that this nanobiosensor could potentially detect miRNA- 128 and distinguish between these two cancers and control samples.
Keywords: Acute Lymphoblastic Leukemia, Hexacyanoferrate, microRNA-128, Nanobiosensor, Reduced Graphene Oxide

Ps-13: Fabrication of Carbon Quantum Dot-based Biosensor for Measurement of A MicroRNA Involved in Acute Lymphoblastic Leukemia

Basir Haghighi N[1*], Basir Haghighi N[2], Mohammad Nejad J[1], Yazdian F[1], Shabani Shayeh J[3], Omidi M[3], Pourmadadi M[4]

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Iran
2. Department of Microbiology, College of Science, University of Tehran, Tehran, Iran
3. Protein Research Center, Shahid Beheshti University, Tehran, Iran
4. Department of Biotechnology, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
Email: niki.basir@gmail.com

Objective: Expression of miRNA-128 plays a significant role in several types of cancers, such as acute lymphoblastic leukemia (ALL), glioblastoma, and hepatocellular carcinoma. Due to the high sensitivity, specificity, user-friendly and rapid characteristics of nanobiosensors, their development could lead to early detection of biomarkers. Carbon quantum dots are non-toxic materials that can decrease the limit of detection and increase signal amplification in nanobiosensors.
Materials and Methods: In this study, an electrochemical nanobiosensor was based on a single-stranded DNA aptamer for the detection of miRNA-128. The basement membrane of the sensor was formed by reduced graphene oxide (RGO)/ gold nanoparticles (AuNPs)/ aptamer; and two metallic carbon quantum dots (CQDs), Fe-CQDs, and Zn-CQDs, were used to amplify the signal in label-free and labeling assays.
Results: The results demonstrate that Fe-CQD performed more efficiently as a label-free agent, and Zn-CQD performed better as a labeling agent. Both methods have excellent selectivity and sensitivity to miRNA-128, with a limit of detection of 0.00973 and 0.00994, respectively, in label-free and labeling methods. Fourier transform infrared (FTIR), X-ray diffraction (XRD), and Transmission Electron Microscopy (TEM) analyses were used to assess the basement membrane characterization, and XRD, FTIR, and Dynamic Light Scattering (DLS/Zeta) were applied to confirm the quality of the synthesized CQDs. The stability and diffusion control analyses were performed in both methods as well.
Conclusion: The findings demonstrate that nanobiosensors have high selectivity and sensitivity for miRNA-128. Both Fe-CQD and Zn-CQD improved the detection of miRNA-128 compared to previous methods and can be used as signal amplifiers in nanobiosensors.
Keywords: Carbon Quantum Dot, Label-Free Sensor, miRNA-128, Nanobiosensor

Ps-14: Expression and Purification of PTPRN Extracellular Domain in Bl21 Bacteria

Dabiri H[1*], Habibi-Anbouhi M[2], Ziaei V[2], Moghadasi Z[2], Hajizadeh-Saffar E[3], Sadeghizadeh M[1]

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. National Cell Bank of Iran, Pasteur Institute of Iran, 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: ehs.hajizadeh@gmail.com

Objective: To target insulin-producing beta cells in type I diabetes, some cell surface antigens are used to generate specific monoclonal antibodies. For this aim, in the first step, the surface antigen is expressed and purified from the appropriate host cells for immunization and the bio-panning process of antibody

production.
Materials and Methods: In this study, the sequence of the PTPRN extracellular domain was extracted from the NCBI database. Codon optimization and gene synthesis was carried out by Gene Cust Company (France). The DNA Fragment was cloned in the Pet 15-b expression vector and transformed into the Bl21 bacteria. After induction by IPTG, the expressed protein was purified by Ni-NTA resin column.
Results: Our results show appeared single 12 kDa protein bands in SDS-PAGE indicating the extracellular domain of PTPRN protein which is seen thicker in stricter conditions during the washing stage.
Conclusion: The purified expressed protein of the PTPRN extracellular domain can be used in beta-specific antibody production procedures.
Keywords: Cell Surface Antigen, Pancreatic Beta Cells, PTPRN Extracellular Domain, Type I Diabetes

Ps-15: Expression and Purification of Anti-IA2 Single Chain Fragment Variable Antibody in HB2151 Bacteria

Dabiri H[1, 2*], Habibi-Anbouhi M[3], Ziaei V[3], Moghadasi Z[3], Hajizadeh-Saffar E[2], Sadeghizadeh M[1]

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, 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. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
Email: ehs.hajizadeh@gmail.com

Objective: To target insulin-producing beta cells in type I diabetes, we developed a single chain fragment variable (scFv) monoclonal antibody against the IA-2 extracellular domain using the phage display technique. To characterization of the scFv affinity and specificity, soluble scFv was needed.
Materials and Methods: In this study, the DNA fragment of anti-IA-2 scFv was digested from the Psex81 phagemid vector and sub-cloned into the POPE101 expression vector. The POPE101-scFv recombinant vector was transformed into the HB2151 bacteria. After induction by IPTG, the scFv protein was extracted by heat shock protocol from the periplasmic space of the bacteria and purified using a Ni-NTA resin column.
Results: Our results show appeared 28 kDa protein band in SDS-PAGE indicating the anti-IA-2 scFv which is seen thicker in stricter conditions during the washing stage.
Conclusion: The purified expressed protein of anti-IA-2 scFv can be used in the characterization procedures of soluble scFv.
Keywords: IA-2 Extracellular Domain, Type I Diabetes, Pancreatic Beta Cells, scFv Monoclonal Antibody

Ps-16: Examining the Potential of Serum Hepcidin as a Biomarker for Iron-Deficient Anaemia

Dayer D*, ASADizadeh S

Cellular and Molecular Research Center, Ahvaz, Iran
Email: asadizadehshahla@gmail.com

Objective: Hepcidine is one of the most significant regulators of the body's iron homeostasis. This study assessed the idea of employing hepcidin as a diagnostic test for iron-deficient anaemia.
Materials and Methods: Forty women with iron deficiency anaemia and forty healthy women participated in case-control research. The serum hepcidin level was evaluated using the ELISA method, and serum ferritin concentrations were determined using the quantitative luminescence method. The serum hepcidin level was evaluated using the ELISA method, and serum ferritin concentrations were determined using the quantitative luminescence method. Pearson's test was used to determine the relationship between hepcidin concentration and ferritin. The Hepcidin cut-off point was determined using the ROC curve.
Results: The average serum level of hepcidin in the group with iron deficiency anaemia was calculated as 9.99±6.10 ng/ml, which showed a significant decrease compared to the healthy group (72.93±46.12 ng/ml) ($p<0.001$). In both studied groups, hepcidin and ferritin serum concentrations showed a significant positive correlation ($p<0.05$). The cut-off point of hepcidin serum concentration for diagnosing iron deficiency anaemia was calculated as 18 µg/ml (95% sensitivity and 92% specificity).
Conclusion: Serum hepcidin concentration can be considered a potential biomarker candidate in diagnosing iron deficiency anaemia.
Keywords: Biomarker, Hepcidin, Ferritin, Iron-Deficient Anaemia

Ps-17: Cryaa: dsRED Zebrafish as A Transgenic Model to Study Eye Disorder

Delkhosh-kasmaie F[1, 2*], Asgari Abibeiglou B[1], Rezaei M[1, 2], Tahamtani Y[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. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Emails: y.tahamtani@royan-rc.ac.ir, mohammad.rezaei@royaninstitute.org

Objective: Structural and functional similarities of zebrafish eyes to human ones and other advantages such as larvae transparency, easy genetic manipulation, and possibility of high throughput drug screening have made it a great model for research in eye's diseases. Crystallins as structural proteins provide transparency and also proper refractive index in the eye lens. Here, we generated a transgenic zebrafish line for crystallins proteins that provides a valuable tool for ocular disorder investigations and drug discovery studies.
Materials and Methods: Tol2 construct was used to generate Tg (cryaa:RFP) transgenic zebrafish. In this technique, the regulatory region of the gene must be inserted upstream of the reporter gene. Transposase mRNA was prepared from pCS2 plasmid using *in vitro* transcription. Wild-type adults were bred and fertilized embryos were collected for co-injection of recombinant plasmid and mRNA. Then, injected embryos were evaluated at 4 days post fertilization (dpf) to select larvae which expressed RFP in their eyes.
Results: Insertion of Cryaa promoter was confirmed by colony PCR and digestion. The quality of mRNA transposase confirmed by gel electrophoreses. A total of 500 embryos were injected and analyzed by fluorescent microscopy and showed that 25 embryos expressed RFP in their eyes.
Conclusion: This transgenic zebrafish model could be used for developmental biology, regenerative medicine as well as drug discovery experiments to study different aspects of eye lens.

Keywords: Drug Discovery, Eye Lens, Ocular Impairment, Transgenic Zebrafish

Ps-18: Different Strategies of Pre-Extracellular Vesicle (EV) Isolation Processing of Plasma Affect The Expression of General EV Markers after Isolation

Farahi F*, Shekari F

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

Objective: Extracellular vesicles (EVs) are a valuable source of biomarkers for the diagnosis of diseases that can be accessed through minimally invasive methods. However, platelet derived EVs and lipoproteins (LPs), which have similar biophysical properties to EVs make downstream applications challenging. Therefore, pre-EV isolation strategies for the separation of EVs are crucial for removing contamination. This study investigated the effects of different pre-EV isolation strategies including freezing steps, centrifugation rounds, and plasma acidification on EV isolation.
Materials and Methods: Blood samples were collected from individuals using tubes containing EDTA anticoagulant. The plasma of the blood samples was separated using different centrifugation rounds of 420g and 2500g and . Second, the samples were divided into two groups. The first group had their EVs immediately isolated after plasma separation, while the second group had their plasmas frozen, and EV isolation was performed after freezing. Third, the plasma samples were divided into two groups. The first group was acidified with PBS+HCl to pH=5.5, and the second group was diluted with PBS. Following a similar EV isolation procedure, the protein concentration of all samples were determined by BCA assay, and protein pattern was checked on SDS-PAGE. Finally, the expression of biomarkers CD81, CD9, CD63, and TSG, was assessed using western blot analysis according to MISEV2018 guideline.
Results: The western blot results showed higher expression in samples whose plasma was separated by 420 g spin compared to samples separated by 2500 g spin, and in samples that were frozen compared to freshly isolated samples. Samples diluted with PBS showed similar results in terms of the expression of EV markers.
Conclusion: The higher expression of the markers in samples that were separated by low-speed centrifugation and samples that were frozen may indicate the presence of more platelet EVs in them. Additionally, evaluating similar results obtained with dilution and acidification suggests that dilution of plasma samples can effectively evaluate plasma EVs.
Keywords: Extracellular Vesicle, Liquid Biopsy, Plasma, Pre-EV Isolation Processing

Ps-19: Physical Properties of Silk Fibroin and Cellulose Nanocrystal Blended Hydrogel for Cartilage Tissue Engineering: Mechanical and Morphological Properties

Farokhi M*, Solouk A, Mirzadeh H

Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: atefeh.solouk@gmail.com

Objective: In this study, a mechanical robust multifunctional hydrogel based on silk fibroin (SF) reinforced by cellulose nanocrystal (CNC) using a Nano filler reinforcement strategy was reported. In addition, horse radish peroxide is utilized as enzymatically crosslinking agent material to form the network. Owing to tyrosine bond formation, and supramolecular interaction between SF and CNC, the prepared hydrogel exhibited satisfying mechanical properties.
Materials and Methods: To prepare SF/CNC hybrid hydrogels, different weight ratios of CNC were added and mixed with SF solution to prepare samples with different concentrations of CNC. The crosslinking process included adding horseradish peroxidase and H_2O_2 (1% Mm).
Results: The result exhibited that the increasing CNC density could elevate the gelation time. In detail, while CNC concentration increases from 0.125 wt% to 0.5 wt%, the density of the di-tyrosine bond increases in this respect, and this difference is significant. CNC size distributions have been measured by DLS and it showed that the average size of nanoparticles is around 250 nm. SEM images showed the morphology of freeze-dried samples of cellulose and cellulose-silk fibroin gels. The cellulose gel is composed of long, interconnected cellulose fibrils forming three-dimensional networks. It exhibited that increasing CNC concentration could enhance the pore size and porosity. As it is calculated, the porosity of the structure is around 70 % which is proper for biomedical applications in cartilage repair. The compressive strength of SF-HG was around 10 kPa and increased to 30 kPa. Biological investigation and observation depict proper cell attachment and cell viability for C28/I2 cells for hydrogels containing cellulose.
Conclusion: CNC could improve the mechanical properties of the hydrogel in a way to be proper for cartilage tissue engineering.
Keywords: Cellulose Nanocrystal, Hydrogel, Mechanical Properties, Silk Fibroin

Ps-20: Effect of Platelet Rich Plasma and Exosomes Secreted by Adipose-Derived Mesenchymal Stem Cells on Restoring Testicular Histology, Size and Weight in NOA Rat Model

Fazaeli H[1*], Sheikholeslami A[1], Davoodi Asl F[1], Sheykhhasan M[1], Naserpour L[1, 2, 3], Kalhor N[1], Fazaeli H[1]

1. Department of Mesenchymal Stem Cells, Academic Center for Education, Culture, and Research ACECR, Qom Branch, Qom, Iran
2. Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom Branch, Qom, Iran
Email: hodafazaely@yahoo.com

Objective: Because non-obstructive azoospermia (NOA) patients are unable to conceive children of their own, their only options are adoption or the use of donated sperm. Therefore, there is an urgent need for additional therapy alternatives for patients with NOA. The present study verified the beneficial effects of platelet rich plasma (PRP) and exosomes secreted by adipose-derived mesenchymal stem cells (AD-Exo) on the testicular histology, size, and weight in busulfan-induced NOA rat model.
Materials and Methods: The exosomes were isolated from the conditioned medium obtained from AD-MSCs. Also, peripheral blood was collected from volunteered individuals and PRP was separated in accordance with the manufacturer's instructions. To induce NOA, two intraperitoneal injections of busulfan (10 mg/kg body weight) were administered within a 21 days interval. Intratesticular injections of 100 microliters of exosome (500 mg/mL), 100 microliters of PRP, and 100 microliters of

PBS was done in AD-Exo, PRP, and sham groups, respectively, at the time points of three days and two weeks after NOA induction. Two months after receiving their last dose, the rats were killed for additional analysis.
Results: AD-Exo and PRP groups alone significantly improved the testicular weight and length compared to the NOA group. However, there was not a significant difference in the testicular width between the AD-Exo and PRP groups. Additionally, when compared to the NOA group, the testicular width was significantly increased by the AD-Exo group alone. Furthermore, testicular alterations show no statistically significant difference between AD-Exo and PRP groups alone compared to the NOA group following PRP/AD-Exo injection.
Conclusion: The study's results validated the beneficial effects of PRP and AD-Exo on testicular histology, size and weight of busulfan-induced NOA models. PRP and exosomes secreted from adipose-derived stem cells may thus be used as a treatment approach that can help patients suffering from NOA.
Keywords: Adipose-Derived Mesenchymal Stem Cells, Exosomes, NOA, PRP

Ps-21: Piezoelectric Evaluation of Poly (L-Lactide) Electrospun Scaffold for Bone Tissue Regeneration

Fotoukian H[1, 2*], Haghbin Nazarpak M[2], Solouk A[1], Nouri A[1],

1. Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
2. New Technologies Research Center (NTRC), Amirkabir University of Technology, Tehran, Iran
Email: haghbin@aut.ac.ir

Objective: Although the piezoelectric characteristics of bone tissue have been widely studied, they are often overlooked in the development of new bone tissue scaffolds, which tend to prioritize the tissue's structural and mechanical properties. Electrospun scaffolds with piezoelectric properties have the potential to replicate the fibrous ECM of bone tissue and facilitate electrical and mechanical stimulation, thereby promoting the regeneration of bone defects. In this study, a poly(L-lactide) (PLLA) electrospun scaffold was fabricated for enhancing bone tissue regeneration.
Materials and Methods: For this purpose, PLLA granules were first dissolved in chloroform and then dimethylformamide was added to the solution system. Electrospinning was performed with a voltage of 18 kV, a distance of 20 cm between the collector and the needle, and a flow rate of 0.7 ml/hour. The nanofibers were characterized using a scanning electron microscope (SEM) and the piezoelectric sensitivity of the electrospun scaffold was measured using a homemade piezo-tester and compared with freeze-dried bone putty (Iran Tissue Production Co.) with the same thickness.
Results: The structure of the electrospun scaffold has a uniform, random and bead-free morphology with a piezoelectric sensitivity of approximately 3.23 ± 0.13 mV/N. The piezoelectric sensitivity of bone putty is equal to 0.15 ± 0.01 mV/N.
Conclusion: The fabricated scaffold is a promising candidate for bone tissue engineering by virtue of its nanofibrous morphology. The PLLA scaffold has piezoelectric properties approximately 21 times that of bone putty and is likely to have a positive effect on cell fate and secretion of growth factors.
Keywords: Bone Tissue Engineering, Electrospinning, Nanofibers, Piezoelectric

Ps-22: Investigation of Differences between Genes and Biological Pathways in Pediatric and Adult Acute Myeloid Leukemia

Ghadimi Nejad Z[1, 2*], Rostami Z[1, 2], Taleahmad S[1]

1. Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Biological Science and Technology, University of Science and Culture, Tehran, Iran
Email: s.taleahmad@royan-rc.ac.ir

Objective: Acute myeloid leukemia (AML) is an aggressive type of leukemia that originates in bone marrow. Hematopoietic stem cells differentiate into lymphoid stem cells which differentiates white blood cells; and myeloid stem cells, which develops red blood cells, platelets and myeloblast that produces granulocytes. Mutation in myeloid stem cells and myeloblasts causes myeloid leukemias. Clinical treatments for this illness would be chemotherapy, hematopoietic stem cell transplant (HSCT), radiation therapy, and targeted therapy.
Materials and Methods: 56 adult samples and 58 pediatric samples from Gene Expression Omnibus were downloaded. Data analysis were done with R; then STRING database, Cytoscape and Gephi were used for protein-protein interactions and finding hub genes. At last enrichment analysis were conducted from EnrichR database.
Results: By looking at Degree, centrality values and eigenvector, we conducted 30 down-regulated hub genes, in which TP53, NOTCH1 and HSPA4 were top three ones; and prolactin signaling, Gastrin signaling, CCKR signaling pathways are top pathways they are involved in. And 16 up-regulated hub genes were conducted, in which RPS15A, RPS21 and NHP2L1 were top three ones, and Thermogenesis and oxidative phosphorylation are top pathways they are involved in.
Conclusion: As suggested in multiple articles, acute myeloid leukemia shows different characteristics in adults and children; so, there must be different therapeutic strategies for each. Knowing differences between genes and pathways in adults and children's patients will lead to more efficient therapies. The main place for down-regulated pathways is nucleus and for up-regulated pathways is Mitochondrial Respiratory Chain Complex.
Keywords: Acute Myeloid Leukemia, Adult AML, Enrichment Analysis, Pediatric AML, Microarray Analysis

Ps-23: Synergistic Effects of Urtica Dioica Hydroalcoholic Extract and Molybdenum Disulfide Nanoparticles on Streptozocine Induced Rin-5f Cell Line

Ghafri F*, Froutan T

Department of Animal Biology, Kharazmi University, Tehran, Iran
Email: fatemhghafari.3070@gmail.com

Objective: Diabetes mellitus (DM) is a complicated metabolic disorder with no definite treatment. Herbal remedies related to the ecosystem of each geographical area have been used to reduce symptoms of various diseases along the time. One on the Medicinal Plants with a wide background in the treatment of diabetes is Urtica diouca (UD)which is known as nettle sting. On the other hand, nanotechnology emerged as a new promising agent in the treatment of various diseases such as Alzheimer

and different types of cancers. In this *in vitro* study we aimed to investigate the possible antidiabetic effects and Biocompatibility of molybdenum di sulfide nanoparticles (MoS2 NPs), one of the less investigated two-dimensional metal nanomaterials, in living cellular environments.
Materials and Methods: The pancreatic beta cell line (RIN-5F) were induced by streptozocine in order to intimate cell's condition under diabetes stress. At first, we obtained the optimal concentration of UD hydro alcoholic extract and MoS2NPs by MTT test. Then in the second phase of study we used ELISA to assay insulin concentration in cell culture medium. We also determined the expression of glucose metabolism-related genes like GCK,GLUT2,INS. Inorder to study the possible cytoprotective effect of the agents we measured BCl2 gene expression too.
Results: According to our results MoS2 NPs and UD hydroalcholic extract concentration shows increasing in insulin secreting levels individually and synergic. And have improved effects on some of glucose metabolism-related genes like GCK,GLUT2,INS and apoptotic genes like BCL.
Conclusion: In conclusion, defined concentration of MoS2 NPs and UD hydroalcholic extract can show synergic antidiabetic effects on some of parameters but have antagonistic effects on the others.
Keywords: Diabetes, Molybdenum Disulfide Nanoparticles, Urtica Dioica

Ps-24: Identification of Hub Genes and Key Pathways in Rheumatoid Arthritis by Bioinformatics Analysis

Ghiasvand M[1*], Taghiyar L[2], Talkhabi M[1]

1. Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, 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: m_talkhabi@sbu.ac.ir

Objective: Rheumatoid arthritis (RA), an autoimmune disease, affects approximately 0.5 to 1% of adults globally. The aim of this study was to investigate hub genes and efficient pathways that may be associated in RA by bioinformatics analysis.
Materials and Methods: The GSE97779 dataset was obtained from the Gene Expression Omnibus database (GEO). The analyses were conducted between 9 samples of macrophages from the synovial fluid of RA patients and five macrophage samples from healthy donor blood samples. Then, differentially expressed genes (DEGs) were normalized using the Transcriptome Analysis Console (TAC). Subsequently, DEGs were discovered with an adjusted P-value of < 0.001 and -2/5<|[log FC]|<2/5. STRING, Cytoscape, and Gephi were used to visualize the protein-protein interaction (PPI) network. Finally, the DEGs were analyzed using the KEGG pathway database.
Results: According to the results 2094 DEGs (1278 up-regulated and 816 down-regulated) were discovered. We found five hub genes included TNF, IL1B, MYC, ITGAM and VEGFA. Also these genes were enriched in significant pathways, including Pathways in cancer, Cytokine-cytokine receptor interaction, Influenza A, Toll-like receptor signaling pathway and PI3K-Akt signaling pathway according to the findings of the KEGG pathway analysis. In addition the DEGs were significantly enriched in the following GO terms (most significant): Cytokine-mediated signaling pathway (GO:0019221), Defense response to virus (GO:0051607) and Positive regulation of cytokine production (GO:0001819) for biological processes.
Conclusion: Consequently, we discovered some significant genes and key pathways that might be used in diagnosis, and therapy of RA in the future.
Keywords: Bioinformatics, Differentially Expressed Genes, Hub Genes, Key Pathways, Rheumatoid Arthritis

Ps-25: Mechanobiological Aspects of Anti-Cancerous Effects of CLA on Hepatocellular Carcinoma Cells

Ghoytasi I[1, 2], Kazemi Ashtiani M[3], Vosough M[2, 4]

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 Regenerative Medicine, Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Huddinge, Sweden
Email: masvos@royaninstitute.org

Objective: Hepatocellular carcinoma is a lethal form of cancer, ranking third in global mortality rates. Recent studies have shown that conjugated linoleic acid (CLA) can inhibit the epithelial to mesenchymal transition (EMT) pathway by increasing the expression level of the HNF4α gene, leading to a reduction in hepatocellular carcinoma phenotypes.
Materials and Methods: This study aims to investigate the effect of treatment with CLA on the cytoskeleton and mechanical deformation of Hep3B cells. The behavior and morphology of Hep3B cells were monitored and evaluated in different control and treatment groups. Confocal microscopy was used to visualize nucleus, actin filaments, and microtubules to investigate possible changes in the cytoskeleton. The migration capacity, morphology, and deformation rate of cells were also evaluated to investigate their behavior and mechanical deformation during treatment.
Results: Results showed that the ratio of corrected total cell fluorescence (CTCF) of actin filaments to microtubules increased from 1.11 to 2.3 in the treatment group after treatment with 150 μM CLA. Additionally, treated cells exhibited reduced migration capacity and decreased size ratio compared to the control cells from 24 hours to 72 hours after treatment. Furthermore, the rate of deformation decreased from 5.84 μm/h to 1.43 μm/h during this period for treated cells.
Conclusion: These findings suggested that treatment with CLA may be a promising modality for inducing MET pathway by altering the mechanical properties of the cytoskeleton.
Keywords: Conjugated Linoleic Acid, Hepatocellular Carcinoma Cells, Mechanobiology of Cancer Cells, MET Induction

Ps-26: Differentiation of Human Induced Pluripotent Stem Cells into Osteoblasts and Adipocytes: A Potential Strategy for Reducing Acute Myeloid Leukemia Severity

Golian H[1*], Nasri S[2], Mirdar-Soltani M[1], Heidari S[3]

1. Department of Biotechnology, Faculty of Sciences and Advanced Biological Technologies, University of Science and Culture, Tehran, Iran

2. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
3. Department of Biotechnology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Email: hamidreza.golian@stu.usc.ac.ir

Objective: Acute myeloid leukemia (AML) is a hematologic cancer caused by abnormal stem cell differentiation, which leads to the accumulation of abnormal cells in the bone marrow. Human induced pluripotent stem cells (hiPSCs) are considered a promising source for producing differentiated cells that can be used in animal modeling, developmental studies, and drug screening. Here, we investigated the ability of hiPSCs to differentiate into osteoblasts and adipocytes as defective cells of the myelocytic lineage and considered their possible usage as barriers in AML metastasis, aggressiveness, or growth rate.
Materials and Methods: HiPSCs were cultured and expanded during several passages. The cells were then cultured for 23 days in a suitable medium for differentiation into bone and fat cells. Differentiated cells were assessed by specific staining and qRT-PCR. 1- Cultivation of embryonic fibroblast cells (MEF) 2- Cultivation of hiPSCs 3- Differentiation of human induced pluripotent cells into bone cells (osteoblasts) 4- Differentiation of human induced pluripotent cells into fat cells (adipocytes) 5- RNA extraction and qRT-PCR
Results: HiPSCs formed bone masses in the bone differentiation medium, which were stained red with alizarin red. In the lipid differentiation medium, lipid droplets accumulated in the cytoplasm of the cells, which were stained red with Oil Red O. Based on the qRT-PCR results, examining the mRNA expression of bone and fat markers in differentiated hiPSCs, genes specific to bone cells, including osteocalcin and osteopontin, and genes specific to fat cells, including LPL and PPAR, were expressed, respectively.
Conclusion: The hiPSC-derived bone and fat cells might be beneficial for potentially postponing AML symptoms and mitigating the intensity of the cancer. These newly differentiated bone cells can replace defective cancer cells, leading to effective hematopoietic compensation and potentially reducing the severity of the disease through these cell modifications.
Keywords: Adipocyte Differentiation, Acute Myeloid Leukemia,

Hematopoietic Stem Cell, Osteoblast Differentiation, Metastasis Inhibition

Ps-27: Design and Fabrication of Highly Conductive PLA-CNT Nanocomposite Electrodes for Biosensing Applications

Hadavand Mirzaei M[1], Molaabasi F[2], Shokati F[2], Zare Y[2*], Iman Shabani[1*]

1. Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
2. Department of Interdisciplinary Technologies, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
Email: shabani@aut.ac.ir, y.zare@aut.ac.ir

Background: Conductive polymer nanocomposites have emerged as a promising platform for the development of highly sensitive and selective biosensors. In this study, we report the design and fabrication of a novel conductive polymer nanocomposite based on polylactic acid (PLA) and carbon nanotubes (CNTs) for various applications.
Materials and Methods: A PLA-CNT nanocomposite film was prepared via a solvent casting method, and the resulting film was cut into small electrodes. The electrodes were characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and electrical conductivity measurements by two-point and Palmsens3 device.
Results: SEM images showed that the CNTs were uniformly dispersed throughout the PLA matrix, forming a well-defined network of conductive pathways. FTIR spectra confirmed the successful incorporation of CNTs into the PLA matrix. The electrical conductivity of the PLA-CNT electrodes was significantly higher than that of pure PLA films, indicating that the incorporation of CNTs into the PLA matrix significantly enhances the electrical conductivity of the nanocomposite film.
Conclusion: The results demonstrate the potential of PLA-CNT nanocomposites as a versatile platform for the development of highly conductive electrodes with excellent properties. In particular, the high sensitivity and selectivity of the electrodes make them suitable for cancer cell detection. The PLA-CNT electrodes offer a promising tool for the early diagnosis and monitoring of cancer cells, which could ultimately contribute to improved patient outcomes and more effective treatment strategies.
Keywords: Biosensors, Carbon Nanotube, Nanocomposite, Polylactic Acid

Ps-28: Encapsulation of Calcium Peroxide Nanoparticles in Silk Fibroin Microparticles to Obtain Sustained Oxygen Release for Tissue Engineering Applications

Haki M*, Moeini M

Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: m.moeini@aut.ac.ir

Objective: The development of tissue engineering approaches requires innovative strategies to provide appropriate oxygen levels to the engineered tissues. Calcium peroxide nanoparticles have been proposed as an oxygen release system, but their rapid degradation and short-term oxygen release limit their efficacy.
Materials and Methods: Here, we present a novel approach that combines calcium peroxide nanoparticles with silk fibroin microparticles carriers to achieve sustained oxygen release.
Results: We demonstrate that silk fibroin microparticles efficiently encapsulate calcium peroxide nanoparticles and significantly slow down the release of oxygen. The slow oxygen release rate is attributed to the microporous structure of the fibroin particles, which effectively controls the water diffusion. We further show that the morphology of microparticles and their oxygen release can be modulated by changing the concentration of silk fibroin, although the effect on oxygen release profile is not substantial. Cellular studies demonstrate that these microparticles significantly improve cell viability and proliferation compared to traditional oxygen delivery systems. In brain tissue engineering, sustained oxygen release enhances brain tissue oxygenation and regeneration, promoting neuronal survival, angiogenesis, and tissue integration.
Conclusion: Overall, our findings suggest that the encapsulation of calcium peroxide nanoparticles in silk fibroin microparticles could be a promising approach to achieve sustained oxygen release in tissue engineering applications and overcome the limitations of traditional oxygen delivery systems. This approach could ultimately help to prevent hypoxia and promote

tissue regeneration.
Keywords: Calcium Peroxide Nanoparticles, Cell Viability, Hypoxia, Silk Fibroin Microparticles, Sustained Oxygen Release

Ps-29: In-silico Analysis to Disclose Key microRNAs and Identification of Protein-Protein Interactions of SRY-box Transcription Factors Involved in The Non-Obstructive Azoospermia

Hasani Mahforoozmahalleh Z[1*], Hashemi Karoii D[2, 3]

1. Department of Microbial Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
2. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: d.hashemi.karoii@ut.ac.ir

Objective: Non-obstructive azoospermia (NOA) refers to the absence of sperm in secondary ejaculation during spermatogenesis. The increase of abnormalities in patients with this disorder made researchers look for new ways of identifying and treating; hence the recognition of its genetic component is demanded as it may simplify an appropriate clinical decision for enhancing fertility. Sex-determining Region Y (SRY)-box (SOX) transcription factor genes are well-known regulators of cell fate decisions during development. They have significant functions and can be a good choice for gene studies involved in non-obstructive azoospermia.
Materials and Methods: In this study, protein-protein interaction (PPI) and microRNA databases are used to predict and find hub genes by Cytoscape software, some microRNA and Enrichr database. After filtering out the hub PPI and microRNA, molecules docking study was performed. We chose SOX5 and SOX8 from 93 Homo Sapiens-related genes found in NCBI's gene database. Genes are enriched and hsa-miR-3681, hsa-miR-4484, and hsa-miR-38 may be associated with NOA.
Results: Five hub genes were identified in the PPI network including ETNK1, MAF, COL2A1, FOXL2, and FEZF2. These genes correlate with SOX5 and SOX8 involved in non-obstructive azoospermia.
Conclusion: By identifying the microRNA interaction, the hsa-miR-3681, hsa-miR-4484, and hsa-miR-38, it may also be possible to determine the activity of the hub proteins network with SOX5 and SOX8 involved in NOA. These miRNA-PPI targets could be valuable biomarkers for NOA.
Keywords: Biomarkers, miRNA, Non-Obstructive Azoospermia, SOX5, SOX8

Ps-30: The Effect of Conditioned Medium from Human Adipose-Derived Mesenchymal Stem Cell on Endometriosis Cell Migration

Hedayatnia H

Department of Biology, Islamic Azad University, Science and Research, Tehran, Iran
Email: hassanhedayatnia_3@yahoo.com

Objective: Endometriosis may be linked to tumor-like traits such as invasion and migration. Given current therapies, the development of new and effective treatments are urgently needed. Human adipose-derived mesenchymal stem cells (ADSCs) and their conditioned medium (ADSC-CM) are rich and easily extractable source of MSCs, thereby offering promising prospects for regenerative therapy.
Materials and Methods: About 2mL of menstrual blood collected from infertile women aged 25-35. Mononuclear cells cultured up to passage 3 and cell surface markers analyzed by flow cytometry. To study the effect of conditioned medium on MenSCs migration, MenSCs seeded in 6-well plates and treated with conditioned medium. Scratch assay was performed by creating scratch in the cell monolayer and incubating for 72 hours and imaging tests were performed at o, 24, 48, 72 hours. To analyze the effect of conditioned medium on MenSCs' migration and invasion, mRNA expression of matrix metalloproteinase-2 (MMP2) and MMP9 genes was studied using real-time PCR.
Results: Migration decreased in the treated group 24 hours after scratching compared to the control group (P=0.0001). Conditioned medium treatment also reduced migration at 46 hours after scratching (P=0.003). Migration decreased significantly (P=0.0001) in the conditioned medium group at 72 hours of scratching. Expression of migration related-genes showed no statistically significant difference in MMP2(p=0.06) after treatment with conditioned medium. Additionally ,MMP9 remained unchanged.
Conclusion: The study shows that conditioned medium could stop metastasis in endometriosis, making it a promising therapy. More research is needed to fully understand its effects.
Keywords: Conditioned Medium, Endometriosis, Mesenchymal Stem Cells (MSCs), Menestrual Blood, Migration

Ps-31: A Computational Simulation to Evaluate Mechanical Modulation of Mesenchymal Stem Cells under Dynamic Culture Condition: Effects of Oscillatory Flow

Jianian Tehrani S[*], Vahidi B

Division of Biomedical Engineering, Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
Email: bahman.vahidi@ut.ac.ir

Objective: In regenerative medicine, techniques which allows the replacement of damaged tissue are a rapidly expanding field of interest. Mesenchymal stem cells (MSCs) have been utilized as a promising alternative solution for the treatment of bone defects. Recent experimental results show that shear stress is one of the dominant mechanical stimuli which facilitates the biological cellular process. By ensuring fully developed laminar shear stress Parallel-plate flow chambers (PPFCs) can simulate fluid flow that MSCs experience in a physiological environment. Currently, there is a lack of understanding in evaluating the effect of the oscillatory fluid flow on the mechanical response of the stem cell. To advance the understanding of bone mechanobiology, in the present study, we aim to use the computational fluid dynamics methods to assess the nature of the mechanical stimulus being applied within a PPFC system as a result of oscillatory fluid flow.
Materials and Methods: In this study, according to a previously published experimental research, a PPFC has been designed in the SOLIDWORKS (V 2021) software with a form of rectangle cube to provide a uniform laminar flow. To this end, the length, width and height are considered to be 77, 30 and 2.5 mm. The properties of the fluid are assumed to be equivalent to water. An incompressible oscillatory flow used to perfuse the bioreactor with a frequency of 1 Hz, was applied to the inlet and a zero-pressure boundary was set at the outlet in the COMSOL (V 6) software.

Results: Through fluid dynamic numerical simulation, the fluid velocity and the wall shear stress (WSS) distribution were studied inside the chamber. It was found that in a steady flow condition with a flow rate of 10 ml/min, the average velocity is 0.001 m/s. Therefore, WSS is in the range of 0.003 to 0.004 Pa, a value which was found to enhance osteogenesis. However, with an oscillatory condition the WSS increases significantly to 0.1 Pa. This simulation further highlights the advantage of using PPFCs in bone tissue engineering.
Conclusion: It can be concluded that the oscillatory flow, comparing to the steady flow plays a prominent role in receiving and inducing the differentiation. In our study, a parallel-plate bioreactor was used to apply fluid flow-induced shear stress, which enhanced the osteogenic differentiation due to the higher WSS compared to the static culture. This evidence suggests that mechanical loading is a key component in bone tissue engineering, given that native bone is constantly subjected to mechanical loading in daily activities.
Keywords: Bone Tissue Engineering, Computational Modeling, Mechanical Modulation, Osteogenic Differentiation

Ps-32: Bioinformatic Assessment of Mitochondrial Content of Extracellular Vesicles Isolated by Different Methods followed by Confirmation

Jodeiry Jabarzade S[1, 2]

1. Department of Developmental Biology, University of Science and Culture, 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: jodeirysheyda@gmail.com

Objective: Extracellular vesicles (EVs) are cell-free systems that exist within a cell, separate from the full-cell system. They can also be byproducts of cells. Due to their ability to facilitate intercellular communication, EVs hold significant importance and influence multiple cellular processes. In the treatment field, EVs can be utilized in various ways, including direct use as a cell substitute, vaccine, or carrier. The isolation methods for each subgroup of EVs differ from one another, and depending on the type of isolation method employed, a specific set of EVs can be isolated and utilized. One particular type of EVs that has recently garnered significant attention from researchers is EVs containing mitochondrial content. These EVs have been explored in both pathogenesis and therapeutic studies. In this research, we have compared the mitochondrial content of different mesenchymal EVs through a meta-analysis of published proteomics data and also we confirmed with bioassays in the laboratory.
Materials and Methods: We utilized available proteomics data from EV proteins, which comprised three subpopulations: High-Speed EVs, Ultracentrifuge-EVs, and Mixed-EVs. We compared their protein profiles. Next, we employed prediction software tools including TargetP, DeepMito, MitoFates, and DeepLoc to identify mitochondrial proteins among the EVs proteins. To confirm our findings, we characterized EVs in the laboratory. The presence of membrane proteins confirmed the presence of EVs based on the MISEV2018guideline. Western Blotting was employed to assess the levels of mitochondrial protein markers, including ATP5A1, Cytochrome C, and Mt-co2, in the isolated EVs. Finally, in the last step, we compared the results and data obtained from the bioinformatics analysis with the *in vitro* data.
Results: In this study, although the number of mitochondrial proteins in different subpopulations was very similar, we observed minor variations in the mitochondria content of High-Speed EVs, which were obtained through centrifugation at 20,000g. We also analyzed two other subpopulations: Ultracentrifuge EVs at a speed of 100,000 g and Mixed EVs through a combination of meta-analysis and laboratory confirmation.
Conclusion: The findings of this study revealed that the number of overlapping genes between mitochondria and HS-EVs was slightly higher compared to other subpopulations of EVs.
Keywords: Extracellular Vesicles, Meta-Analysis, Mesenchyme, Mitochondria

Ps-33: Investigating The Effect of Direct Co-Culture of Patient-Derived Multiple Myeloma Cells with Wharton-Jelly-Derived Mesenchymal Stem Cells in Gel-Based And Gel-Free Culture Conditions

Jomehpour karizaki M[1*], Vahdat S[2], Abroun S[3], Mousavi SA[4]
1. Department of Hematology, Tarbiat Modares University, Tehran, Iran
2. Applied Cell Sciences Division, Department of Hematology, Tarbiat Modares University, Tehran, Iran
3. Department of Hematology, Tarbiat Modares University, Tehran, Iran
4. Research Institute of Hematology, Oncology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran
Emails: s_vahdat@modares.ac.ir, a_mousavi@tums.ac.ir

Objective: Finding new therapeutics for multiple myeloma (MM) can enhance MM cancer research. Wharton's Jelly-derived mesenchymal stem cells (WJ-MSCs) have tumoricidal properties and their effect on MM cells could be examined. As most cancer studies are conducted on two-dimensional (2D) cultured cell lines, their results rarely translate to clinical studies. In the present study, it was tried to recapitulate the three-dimensional (3D) MM bone marrow (BM) microenvironment in order to investigate the effect of WJ-MSCs on patient-derived myeloma cells in comparison with the U266 MM cell line.
Materials and Methods: Mononuclear cells (MNCs) were isolated from BM samples of MM patients. Co-culture of MNCs or U266 cells with WJ-MSCs was performed in two culture conditions: gel-free (2D) and gel-based (3D) cultures. The effect of co-culture in culture conditions on the survival of myeloma cells (both primary cells and the U266 cell line) was assessed on day three post-culture using flow cytometry.
Results: Co-culture of U266 cells with WJ-MSCs in 2D and 3D culture conditions resulted in increased and decreased death of cells (PI+ cells) at day three post-culture, respectively. The average percentage of CD138+/PI+ population of patients' MNCs was increased in the co-culture group compared to the non-co-culture control group, in 2D culture conditions. However, the percentage of CD138+/PI+ cells in 3D co-culture of MNCs with WJ-MSCs showed patient-based distinct patterns.
Conclusion: We found that culture conditions and inter-individual differences affected cell responses to the same treatments. We found that cell lines and primary cells responded differently, emphasizing the need for a more accurate disease microenvironment.
Keywords: Multiple Myeloma, Wharton-Jelly Mesenchymal Stem Cells, Three-dimensional Culture Condition

Ps-34: RGC-5: A Reliable Tool for Studying Retinal Gan-

glion Cells

Kavianpour K

Department of Stem Cells and Developmental Biology,, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: l.satarian@royan-rc.ac.ir, f.khosrobakhsh@uok.ac.ir

Objective: Retinal ganglion cells (RGCs) are essential neurons responsible for transmitting visual information from the retina to the brain. Understanding the function and vulnerabilities of RGCs is crucial in various visual disorders. However, studying primary RGCs poses challenges due to their limited quantity and purification difficulties. An alternative is to use cell lines, such as RGC-5, for investigating RGC properties.
Materials and Methods: In this study, we demonstrated that the RGC-5 cell line can be a reliable choice for studying RGCs, despite the absence of some RGC markers like homeobox/POU domain protein 3A (BRN3A), and RNAbinding protein with multiple splicing (RBPMS). RGC-5 cells were cultured using DMEM/High glucose and fixed with paraformaldehyde to preserve their structure. Following permeabilization and blocking steps, the cells were incubated with primary and secondary antibodies conjugated to fluorescent markers.
Results: The results showed that RGC markers, including BRN3A and RBPMS, were well expressed in the RGC-5 cell line. Additionally, the presence of Map2 and Beta III tubulin indicated neuronal characteristics, while the absence of Nestin suggested a lack of pre-differentiation markers.The findings demonstrate that the RGC-5 cell line retains important features of RGCs, despite some marker variations. This cell line can serve as a valuable tool for studying RGC properties and mechanisms in different diseases. Further investigations utilizing the RGC-5 cell line can provide insights into the dysfunction and degeneration of RGCs, aiding the development of diagnostic and therapeutic approaches for visual impairments.
Conclusion: By leveraging the RGC-5 cell line, researchers can overcome the limitations associated with primary RGC cultures and gain a better understanding of the intricate processes underlying RGC-related diseases such as Leber hereditary optic neuropathy (LHON) and glaucoma. The study highlights the importance of utilizing reliable cell lines to advance our knowledge of RGC biology and contribute to improved treatments for vision-related conditions.
Keywords: Retinal Ganglion Cell, RGC-5, BRN3A, RBPMS

Ps-35: Rotenone: A Challenging Option for Modeling Leber Hereditary Optic Neuropathy

Kavianpour K*

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran
Email: l.satarian@royan-rc.ac.ir, f.khosrobakhsh@uok.ac.ir

Objective: Leber hereditary optic neuropathy (LHON) is a rare genetic disorder characterized by progressive vision loss due to mitochondrial dysfunction. Retinal ganglion cell (RGC) apoptosis and reduced retinal tissue thickness are key indicators of LHON. Rotenone, a mitochondrial complex I inhibitor, is commonly used to create LHON animal models, inducing retinal thinning and RGC apoptosis. Horizontal cells, found in the Outer Plexiform Layer (OPL) of the retina, play a crucial role in modulating signal transmission between bipolar cells and photoreceptor cells. To investigate the status of horizontal cells in the OPL, researchers use a calcium-binding protein called Calbindin, which is involved in various functions within the eye, including calcium signaling and synaptic transmission.
Materials and Methods: In this study, rotenone was intravitreally injected into wild-type mice, resulting in visual impairments and an increase in OPL thickness, consistent with previous research. The expression of Calbindin was significantly reduced in the rotenone-treated group, indicating damage to horizontal cells. Moreover, increased expression of Glial Fibrillary Acidic Protein (GFAP) in the OPL suggests the presence of inflammation and tissue damage.
Results: The findings suggest that rotenone has a detrimental effect on the OPL, particularly on horizontal cells. This damage disrupts the normal signal transduction process and may contribute to the decrease in visual function observed in LHON.
Conclusion: The study highlights the importance of further exploring the impact of rotenone on the OPL and its implications for understanding the mechanisms of LHON.
Keywords: Horizontal Cells, Leber Hereditary Optic Neuropathy, Outer Plexiform Layer, Rotenone

Ps-36: Adipose-Derived Stem Cells Application on Cartilage Repair of Sheep

Khanzadeh Safarloo A[1*], Yaghoobzade A[2], Nikzad S[3]

1. Department of Paramedical Science, Tabriz Azad University of Medical Sciences, Tabriz, Iran
2. Department of Biological and Medical Sciences, Montreal, Canada
Email: armankhanzadeh.7@gmail.com

Objective: The goal of this study has been to evaluate adipose tissue derived stem cells (ADSC) from infrapatellar fat pad and characterize their cell surface markers using anti-human antibodies, as adipose tissue derived stem cells (ADSCs) have great potential for cellular therapies to restore injured tissues.
Materials and Methods: Adipose tissue was obtained from infrapatellar fat pad of sheep. Surface markers evaluated by flow cytometry. In order to evaluate cell adhesion, the Polycaprolactone (PCL) was sterilized under Ultraviolet (UV) light and about 1×10$ cells were seeded on PCL. Then, ASCs- PCL construct were evaluated by Scanning Electron Microscopy (Mira3 Te Scan, Czech Republic).
Results: We showed that adipose tissue derived stem cells (ADSCs) maintain their fibroblastic-like morphology during different subcultures and cell adhesion. They were positive for CD44 and CD90 markers and negative for CD31 and Cd45 markers by human antibodies.
Conclusion: Our results suggest that ASCs surface markers can be characterized by anti-human antibodies in sheep. As stem cells, they can be used in tissue engineering.
Keywords: Adipose tissue, ADSCs, PCL, Stem cells, Tissue Engineering

Ps-39: Effect of Prenatal Inhalation Exposure of Wistar Rats to Crude Oil Vapor on Osteogenic and Adipogenic Differentiation of Fetal Mesenchymal Stem Cells

Khazaeel K[1*], Sadeghi A[1], Tabandeh Mohammad R[2], Nejaddehbashi F[3], Ezzati Givi M[4]

1. Department of Basic Science, Division of Anatomy and Embryology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Cellular and Molecular Research Center, Medical Basic Sciences Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
Email: k.khazaeil@scu.ac.ir

Objective: It has been indicated that crude oil vapor (COV) induces tissue damage by several molecular mechanisms. However, data related to the adverse effects of crude oil pollutants on stem cell fate and differentiation are very limited. This study aimed to investigate the effect of COV on the differentiation of fetal mesenchymal stem cells (fMSCs) following prenatal exposure.
Materials and Methods: Twelve pregnant Wistar rats were equally divided into 2 groups including the control (Normal saline was given orally from 0 to 15 days of gestation) and the COV group (Rats were exposed to COV from 0 to 15 days of gestation). The inhalation method was used to expose the rats to crude oil vapors for 5 hours daily. After treatments, fMSCs were isolated from fetuses using enzymatic digestion and they were differentiated into the osteoblastic and adipogenic lineages. Cell proliferation, osteogenic and adipogenic differentiation markers and the phosphorylation of PI3K and ERK1/2 signaling proteins were evaluated in differentiated cells.
Results: The results of the protein levels of p-PI3K and p-ERK1/2 in osteogenic and adipogenic differentiated fMSCs revealed that exposure to COV significantly increased the level of p-ERK1/2 and p-PI3K as compared to the control ($P<0.05$). Moreover, our findings indicated that prenatal exposure to COV significantly reduced Runx2, BMP-6, osteonectin, and ALP expression in osteogenic differentiated fMSCs compared to the control group ($P<0.05$). Also, COV significantly reduced the expression of the PPARγ and CREBBP genes in fMSCs differentiated into adipocytes compared to the controls ($P<0.05$).
Conclusion: In conclusion, our results indicated that prenatal exposure to COV impaired fMSCs differentiation by regulating ERK1/2 and PI3K signaling pathways and decreasing the expression of genes involved in osteogenesis and adipogenesis.
Keywords: Crude Oil Vapor, Fetal Mesenchymal Stem Cell, Rat, Fetus

Ps-38: Fabrication and Evaluation of Antioxidant Drug-Loaded Hyaluronic Acid Microbeads for Cell Delivery Applications

Khodaman M*, Daraeinejad Z, Shabani I

Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: shabani@aut.ac.ir

Objective: One of the main approaches for the regeneration of damaged tissues is cell therapy. However, due to the harsh environment and presence of reactive oxygen species (ROS) in damaged tissues, a significant number of cells die after transplantation. Therefore, using antioxidant drug-loaded carriers can facilitate harsh environments and reduce ROS. In this study, hyaluronic acid microbeads were loaded with an antioxidant drug for cell delivery to increase the survival rate of cells.
Materials and Methods: Hyaluronic acid microbeads were prepared by dropping hyaluronic acid solution with a syringe pump in a crosslinker solution. Divinyl sulfone was used as a crosslinker to strengthen and improve the mechanical properties of microbeads. During the preparation of the hyaluronic acid solution, the antioxidant drug was added to the content. Microbeads' morphology was characterized by scanning electron microscopy (SEM) and the presence of the crosslinker residual and drug was analyzed with Fourier-transform infrared (FTIR) spectroscopy. Furthermore, the drug release rate from microbeads was investigated by ultraviolet-visible (UV-Vis) spectroscopy.
Results: Based on SEM results drug-loaded hyaluronic acid microbeads had uniform size distribution in the micrometer range. The lack of presence of crosslinker residual and the presence of the drug in the polymeric carriers were confirmed with FTIR after the washing process. UV-Vis spectroscopy showed a slow-release profile without any initial burst release.
Conclusion: Antioxidant drug-loaded hyaluronic acid microbeads due to their biocompatibility and injectability are suitable carriers to increase the survival rate of cells for cell delivery applications.
Keywords: Antioxidant Drug, Cell Delivery, Hyaluronic Acid, Microbeads

Ps-39: Due to Insolubility, Curcumin Partially Was Able to Compensate Oxidative Stress Induced by Di-2-Ethylhexyl Phthalate to Improve Osteogenic Differentiation of Bmscs

Lak M*, Abnosi MH

Department of Biology, Faculty of Basic Sciences, Arak, Iran
Email: mahjobe.lak.1372@gmail.com

Objective: Since Di-(2-ethylhexyl) phthalate (DEHP) is used in polyvinyl chloride products, it has been found to reduces osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) due to induction of oxidative stress. Curcumin, having antioxidant properties, was proposed to compensate adverse effects of DEHP.
Materials and Methods: BMSCs were extracted from Wistar rats and treated with DEHP (100 μM) and curcumin (0.1 μM) individually and simultaneously in osteogenic media for 20 days. The viability, total protein concentration, bone matrix production, ALP activity, MDA level, total antioxidant capacity, activity of superoxide dismutase and catalase and expression of osteogenic related gene were evaluated.
Results: Following DEHP treatment, we observed a significant decrease in bone matrix production based on alizarin red and calcium analysis. On the other hand, the expression of ALP, RUNX2 and alkaline phosphatase enzyme activity decreased. In addition, MDA increased significantly, while the activity of antioxidant enzymes and the level of total antioxidant decreased. Although, simultaneous treatment showed curcumin could improve the toxic effect of DEHP but could not completely compensate its adverse effect compared to the control group.
Conclusion: AS curcumin is highly insoluble in aqueous situation, we propose its encapsulation in delivery system to improve its antioxidant action.
Keywords: Antioxidant, BMSCs, Curcumin, DEHP, Osteogenic

Ps-40: Developing an Osteochondral Construct Using Mesenchymal Stem Cell Sheet Technology

Leisi Mehrabani F[1*], Hosseini S[2], Baghaban Eslaminejad M[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 Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, 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: eslami@royaninstitute.org

Objective: Degenerative diseases and trauma are to blame for the prevalence of combined cartilage-subchondral bone defects and articular cartilage defects. New strategies utilizing stem cell sheets have emerged to enhance the regenerative process of cartilage and bone. Stem cell sheets' cell-to-cell connection, enhancement of extracellular matrix, and scaffold-free nature make them ideal for regenerative medicine. This study aims to develop multi-layered constructs using osteogenic and chondrogenic differentiated mesenchymal stem cell sheets for regeneration of osteochondral defects.
Materials and Methods: MSCs were isolated from rat bone marrow and expanded through frequent passages. Passages 3–6 were used to generate cell sheets by culturing cells in 12-well culture plates. After reaching over-confluency, osteogenic and chondrogenic differentiation were induced. The sheets were harvested mechanically by scraping the cells after 21 days and layered the chondrogenic sheet on top of the osteogenic sheet to generate a multilayer structure. We used gelatin hydrogel microbeads and hyaluronic acid between each cell layer to overcome stockpile limitations. Cell viability was assessed at days 1 and 7. Tissue formation was assessed by histological analysis including hematoxylin & eosin (H&E), Alizarin Red-Alicen Blue, Safranin O-Fast Green staining and DAPI. The real-time polymerase chain reaction (RT-PCR) was also used to evaluate the gene expression level of osteogenic and chondrogenic markers.
Results: Histological analysis showed that a multilayered osteochondral structure was created from differentiated stem cell sheets. The construct thickness was approximately 201.45 μm with two distinct cartilage- and bone-like tissues. The osteochondral construct with hydrogel had higher cell viability than the control group at day 1 and 7. According to qRT-PCR results, the expression level of Col1/Col2/Aggrecan/Osteocalcin increased in the multilayer osteochondral structure with hydrogel.
Conclusion: In conclusion, our data demonstrated that stem cell sheet technology can develop a 3D viable scaffold-free multilayer construct with distinguishable osteogenic and chondrogenic layers.
Keywords: Osteochondral Construct, Mesenchymal Stem Cells, Stem Cell Sheets, Osteogenesis, Chondrogenesis

Ps-41: FACS-Based Screening and UCOE Combined Strategy Accelerates Isolation of High-Producing Cells and Improves Recombinant Protein Productivity in Chinese Hamster Ovary Cells

Lohrasbi R[1, 2*], Daneshipour A[2], Jazayeri Seyede H[3], Halfinezhad Z[2], Norouzi F[2], Abolghasemi Dehaghani S[2], Abd Emami B[2], Dalman A[4], Gharanfoli M[2], Amiri-Yekta A[2]

1. Department of Molecular Cell Biology-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. Faculty of Advanced Sciences and Technology, Tehran Medical sciences, Islamic Azad University, Tehran, Iran
4. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: amir.amiriyekta@gmail.com

Objective: Nowadays, improvement in throughput and safety of biopharmaceuticals with low costs are the priority of recombinant protein production. Utilizing powerful Fluorescence-activated cell sorting (FACS) technique and reporter genes such as enhanced green fluorescent protein (EGFP) provides a quite specific screening and isolating high-productivity cells in the shortest possible time. Furthermore, considering that the random integration procedure has been widely applied to achieve high-producing cells, using genetic regulatory elements like the ubiquitous chromatin opening element (UCOE) tends to be effective in increasing recombinant protein production. Here, we aimed to perform FACS to accelerate high-producing cells selection, and utilize the UCOE to elevate the production rate of CHO DG44 cell line.
Materials and Methods: In the current research, a codon-optimized Darbepoetin alfa (DPO) sequence, based on CHO codon usage, was synthesized, and cloned into the pOptiVECTM and UCOE-containing plasmid, CET 1019 HD-hygro. The cloning process was confirmed by enzymatic digestion, PCR, and followed by Sanger sequencing. Subsequently, linearized gene cassettes that entailed DPO-LoxP-IRES-EGFP-LoxP-IRES-DHFR were stably transfected into the CHO DG44 cells (1.5×10^6 cells/well) through the FreeStyleTM Max reagent. Due to the presence of DHFR sequence in cassettes, on day two of post-transfection, both cell lines were resuspended in CD OptiCHOTM medium for selection purposes. Then, vector integration in the CHO genome was confirmed by PCR and also the transfected positive cells were assessed by fluorescence microscope. EGFP was applied as a selection marker to sort high-level EGFP-producing cells in pOptiVEC-DPO and CET 1019 HD-DPO populations by FACS. Ultimately, DPO expression level was evaluated by RT-qPCR, western blotting, and ELISA in 5 days of cultured, sorted, and unsorted cell lines.
Results: Appraising the present research data demonstrate that all UCOE-containing cell pools indicate higher DPO expression level in comparison with non-UCOE populations. Besides, enrichment of high-producing cells through FACS leads to about 1.5-fold improvement of DPO density in sorted cell lines than unsorted ones. Overall, target protein concentration in isolated CET 1019 HD-DPO cells was dramatically increased compared to control population, unsorted pOptiVEC-DPO, due to the presence of UCOE and sorting effect.
Conclusion: Using FACS as a high-throughput screening strategy to sort high-producing cells and also utilizing UCOE to enhance transgene expression, provides an effective approach to obtaining high-expression clones in a short time, moreover prominently increases recombinant protein production yield.
Keywords: Cell Sorting, CHO DG44, Darbepoetin Alfa, FACS, High-Producing Cell Line

Ps-42: Macrophage Cell Morphology-Imprinted Substrates Can Modulate Mesenchymal Stem Cells Behaviors and Macrophage M1/M2 Polarization for Wound Healing Applications

Majidi M[1*], Pakzad S[2], Salimi M[3], Azadbakht A[4], Bonakdar Sh[5], Gholipourmalekabadi M[1], Hajighasemlou S[2]

1. Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Food and Drug Laboratory Research Center, Food and Drug Administration, Iran Ministry of Health and Medical Education, Tehran, Iran
3. Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Biomedical Engineering, Central Tehran Branch, Islamic Azad University, Tehran, Iran
5. National Cell Bank, Pasteur Institute of Iran, Tehran, Iran
Email: mazaher.gholipour@gmail.com

Objective: Stem cells and macrophages (MQs) are the most important cells in the normal wound-healing process. It is well understood that topological cues and mechanical factors induce different responses in stem cells and MQ by influencing their shape, cytoskeleton, proliferation, migration, and differentiation, which play a key role in the wound healing process. On the other hand, the polarization of MQ from pro-inflammatory (M1) to pro-healing (M2) phenotypes has a critical role in the acceleration of wound healing.
Materials and Methods: In this study, the morphology of different macrophage subtypes (M0, M1, and M2) was imprinted on a silicon surface (polydimethylsiloxane (PDMS)) to prepare a nano-topography cell-imprinted substrate with the ability to conduct cell fate on the mouse adipose-derived stem cells (ADSCs) and RAW264.7 monocyte cell line (MO). MTT, LAL, AFM, fluorescent microscopy, SEM, RT-PCR, ELISA, and flowcytometry assays helped us to understand if the cell shape microstructure promoted the MQ phenotypes according to the specific shape of each pattern.
Results: MO grown on M2 morphological patterns showed a significant increase in the expression and secretion of anti-inflammatory cytokines compared with M0 and M1 patterns. The ADSCs on the patterned PDMS exhibited remarkably different shapes from no-patterned PDMS. The ADSCs homing in niches heavily deformed the cytoskeletal, which is probably why the gene expression and phenotype unexpectedly changed.
Conclusion: In conclusion, the topography can modulate the MO in a pattern-dependent manner and weekly the ADSCs fate. Wound dressings with M2 cell morphology-induced surfaces are suggested as excellent anti-inflammatory and anti-scarring dressings.
Keywords: Cell-Imprinting, Macrophage, M1 & M2 Macrophage, Topography, Wound Dressing

Ps-43: SHH-Overexpressing Bone Marrow-Mesenchymal Stem Cells to Improve The Limb Regeneration

Mashayekhi Mazar F[*], Taghiyar L, Hesaraki M, Baghaban Eslaminejad M

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

Objective: Limb/digit regeneration potency in mammals is limited due to the absence of blastema cells as the key necessary factor for regeneration. One of the critical genes of blastema with an essential role in limb regeneration is the sonic hedgehog (SHH) gene. This study aimed to create a novel blastemal-like cell (BlCs) as an alternative cell source for blastema by overexpression of the Shh gene in human bone marrow mesenchymal stem cells (hBMSCs) to promote regeneration in mice with amputated digit tips.
Materials and Methods: The hBMSCs were isolated from human bone marrow. The cells were characterized for differentiation capacity to skeletal lineage (bone, cartilage, and adipose) and the surface biomarker. The non-integrative plasmid transfection method using the HEK293 cells was applied to transfer the SHH (coupled with the GFP gene) to hBMSCs. The analysis of transduced cells was checked by immunocytochemistry (ICC) and Real-time PCR following GFP-positive cell sorting by flow cytometry. Finally, the regenerative potential of BlCs was measured by transplantation into amputated digit tips in mice.
Results: The identification of hBMSCs was confirmed with differentiation into skeletal lineages, and positive expression of biomarkers ≥95%. Subsequently, the expression of shh and key downstream genes of MSX1, Bmp2, and Fgf8 was confirmed in pure-sorted GFP-positive cells by ICC and RT-PCR. More importantly, BlCs increased mice digit tip regeneration by new bone and nail formation. In contrast, non-transduced hBMSCs formed abnormal bone and nails, and in the sham group, the presents of new tissue weren't observed.
Conclusion: Our results provide evidence of the successful limb regeneration with the SHH overexpression in hBMSCs using a non-integrative viral method as a safe method for clinical application. So, BlCs are appropriate transduced cells for promoting human limb regeneration after clinical verification.
Keywords: Digit tip Regeneration, Mesenchymal Stem Cells, SHH Overexpression, Non-integrative Plasmid, Transduced Cells

Ps-44: Investigating The Relationship between Serum Estradiol and Expression of Transforming Growth Factor Beta (TGFβ), Interleukins (IL)-4, IL-5, IL-12, Cytokine Genes in Pregnant Mice, with A Comparative Analysis of Asthmatic Pregnant Mice

Mehrpour M[1*], Ehsani E, Athari SS[2], Eydi A[3]

1. Department of Stem Cells and Developmental Biology, Islamic Azad University of Sciences and Research, Cell Science Research Center, Royan Institute, Tehran, Iran
2. Science Committee , Zanjan University of Medical Sciences, Zanjan, Iran
3., Islamic Azad University of Sciences and Research, Tehran, Iran
Email: Maryammehrpur71@gmail.com

Objective: Asthma is known as a respiratory disease by increasing the responsiveness of the respiratory network to a group of stimuli. Inflammation plays an essential role in the pathophysiology of asthma, and with the reaction of various immune cells and mediators, it leads to bronchial inflammation, airway obstruction, and clinical manifestations of cough, wheezing, and short-winded. Previous studies have mentioned the effect of sex hormones and inflammatory mediators in this field.

Materials and Methods: In this experimental study, 40 Balb/c mice) 6-8 weeks old (with a weight range of 180-200 gr were used. Mice were randomly divided into 4 groups: healthy (negative control), healthy asthmatic (positive control), pregnant, and pregnant asthmatic (n=10). To induce asthma in animals, an inhalation injection of albumin aerosol was used. To airway responsiveness and increase in eosinophil, Interleukin 4 (IL-4), Interleukin 5, and Interleukin 12 levels were investigated. Eosinophilic inflammation was also investigated by Broncho- alveolar fluid lavage and uterine tissue.
Results: The results of the present research showed that there is a significant relationship between the increase of estradiol hormone and changes in lung function indicators in the group of asthmatic pregnant mice compared to the negative control group, which indicates the dominant role of estradiol sex hormones in the occurrence of the Allergic asthma disease. A significant increase in the level of estradiol hormone was observed in the group of pregnant mice compared to the control group. In the lavage fluid and uterine tissue, it shows a significant relationship between IL-12 expressing cells in mice with asthma compared to the control group. The increase in the level of IL-5 in mice with asthma compared to the control group can also be seen in the results obtained from lung and uterine tissue in this research. A decrease in the level of the TGF-β factor was observed in the positive control group compared to the negative control group due to the anti-inflammatory effect of this factor.
Conclusion: The results showed that changes in the levels of IL-12,4,5 and TGF-β play an important role in the pathology of asthma and airway obstruction. There was a positive relationship between the increase in the level of estradiol hormone and the increase in pulmonary function indicators, indicating the destructive effect of sex hormones in reducing pulmonary function and increasing allergic diseases such as asthma. In the uterine tissue and lavage fluid, cytokines are involved in the process of pregnancy and pregnancy, and this can be seen in the increase in the level of IL-12,4,5 and TGF-β in the pregnant mice group compared to the control group.
Keywords: Asthma, IL-12,4,5, Mice, Pregnancy, TGFβ

Ps-45: Identification and Validation of Gene and MiRNA Expression Profiles for Early Detection of Alzheimer's Disease Using in Silico Analysis and QRT-PCR Technique

Miri MS*, Taleahmad S

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: s.taleahmad@royan-rc.ac.ir

Objective: Alzheimer's is one of the most important causes of disease and death in the world, which has been cited as the sixth cause of death worldwide. Early diagnosis of AD is an important goal. Changes in miRNAs expression, as a result of cellular damage and brain damage, can be identified in CSF and in blood/serum plasma. Therefore, the search for miRNAa in body fluids can be a powerful tool for diagnosing neurological diseases. The study aims to identify and analyze genes with differential expression associated with AD and to examine the expression profiles of blood and brain cells using In silico analysis techniques. Finally, the differentially expressed genes were validated on blood samples of 15 patients with Alzheimer's and 15 non-Alzheimer's people as a qRT-PCR technique. The importance of our issue is that AD disease deaths have increased by 89% in recent years, while deaths of other major diseases such as heart disease, stroke, breast cancer, and prostate and AIDS in the same timeframe have declined.
Materials and Methods: The method of this research and analysis was done using bioinformatics data and Real-time PCR techniques.
Results: Our analyses revealed 58 differentially expressed genes (DEGs) and 8 differentially expressed miRNAs (DEMs) in AD. Among the DEMs, four miRNAs (hsa-miR-5001-3p, hsa-miR-450b-5p, hsa-miR-659-5p, hsa-miR-3916), showed the highest increase in expression in AD. The study also identified three genes (RPE65, NLG4X, GRAMD2A) that were significantly different in the AD group in the initial screening and were found to be related in the SH-SY5Y cell line.
Conclusion: Overall the study provides valuable insights into the identification and validation of gene and miRNA expression profiles for early detection of AD using in silico analysis and qRT-PCR techniques. The finding of this study could potentially contribute to the development of new biomarkers and therapeutic targets for AD.
Keywords: Alzheimer's Disease, Biomarker, MicroRNA, RNA Seq, Transcriptome

Ps-46: Fabrication and Characterization of Hyaluronic Acid Coated Electrospun Chitosan-Based Nanofibers with Resveratrol and Adipose Derived Stem Cell for Wound Healing Application

Moghaddam A[1, 2*], Orazizadeh M[1, 2], Nejaddehbashi F[2], Bayati V[1, 2]

1. Department of Anatomical Sciences, School of Medicine, Ahvaz, Iran
2. Ahvaz Jundishapur University of Medical Sciences, Cellular and Molecular Research Center, Ahvaz, Iran
Email: asmamogomhaddam1371@gmail.c

Objective: Wound healing is a complicated cellular process involving various mechanisms getting it intricate for designing a scaffold for therapeutic applications. This study deals with the designing and development of electrospun chitosan-polyethylene oxide nanofiber wound dressing for capable simultaneous drug delivery. Hyaluronic acid and resveratrol were chosen for coating on chitosan based nanofibers for their efficacy in immunoprotection and pro-angiogenic activities respectively.
Materials and Methods: In this experimental study, an electrospun nanofibrous mat composed of CS and subsequent coating by HA was prepared. Structural, mechanical, and biological properties of nanofibers were evaluated using SEM, FTIR, tensile testing, TGA, *in vitro* release study and human fetal fibroblasts seeding assay, and then used *in vivo* as skin substitutes in a male rat wound model.
Results: According to *in vitro* results, cells adhered and proliferated in all scaffolds. However, cells deep into the scaffold were more detected in the CS/HA and CS/HA/RS scaffolds. In *in vivo* tests CS/HA/RS with AD-MSC scaffold had the highest impact on the healing process by increasing the granulation of wound tissue and enhancing the proliferation and re-epithelialization of the wound.
Conclusion: The electrospun CS/HA/RS with AD-MSC scaffold could be considered as a promising type of wound dressing in wound healing process *in vivo*.
Keywords: Chitosan, Electrospinning, Hyaluronic Acid, Resveratrol, Wound Healing

Ps-47: Functional Analysis of Mitochondrial Content in Different Subpopulations of Extracellular Vesicles derived from Mesenchymal Cells on The Growth of Breast Cancer Cells

Moradpour N[1, 2]

1. Department of Developmental Biology, University of Science and Culture, 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: moradpourniloufar@gmail.com

Objective: Nowadays, extracellular vesicles (EVs) can be used both directly and as drug carriers in various therapeutic research. EVs are a heterogeneous collection of different types of vesicles produced by a cell, and depending on the kind of isolation method, we may select a set of these EVs. One of the new types of EV population that have recently attracted a lot of attention is those EVs that contain mitochondrial content. Research has shown that this group of EVs plays a role in pathogenesis and can be used in therapeutic research.
Materials and Methods: In this research, we first isolated EVs by two methods (20k g and 110K g) and confirmed their identity according to the MISEV2018 guideline by western blotting, electron microscopy, and dynamic light scattering. Then, by examining the expression of mitochondrial proteins such as ATP5A1, mt-CO2, and Cytochrome C, the difference between these EVs is determined based on the level of mitochondrial content. We also isolated mitochondria organelle from mesenchymal stem cells and confirmed their identity by the western blot method. In the next step, we treated the MCF7 breast cancer cell line with the different EVs and pure mitochondria and investigated the effect on the growth and proliferation of cancer cells.
Results: Characterization of EVs isolated by 20K and 110K methods confirmed their identity as EVs, however, molecular analysis revealed distinct differences between the two EV subpopulations. Mitochondria were also isolated from the cells and the presence of mitochondrial proteins was confirmed by western blotting. Differences were observed in the level of mitochondrial proteins between the two EV populations which suggested potential variations in their cellular effects.
Conclusion: This is the first report that shows the differential mitochondrial content of two commonly used populations of EVs. More studies are required to pave the road toward the therapeutic application of EVs with mitochondrial contents.
Keywords: Extracellular Vesicles, EVs, EV Population, Mitochondria

Ps-50: Inhibition OF TGF-β Signaling Pathway Improves Reconstruction of Seminiferous Tubule-Like Structure from Dissociated Testicular Cells

Moraveji F*, Esfandiari F, Ghanian M*, 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: Moraveji_f@yahoo.com

Objective: Testicular organoids (TOs) appear to be a promising research model for drug screening and studying human infertility. Several attempts tried to reconstruct testes from testicular cells *in vitro*; however, only limited organization beyond seminiferous cord formation has been achieved. The objective of this study is to assess the effect of TGF-β inhibitors on organoid formation and progression of spermatogenesis in one of the best-known TO culture systems.
Materials and Methods: To establish a TO culture, isolated germ cells from immature mouse testis (14-15 day old) were cultured in a three-layer gradient system (3-LGS) based on Matrigel. On day 7, the TOs were treated with inhibitors of TGF-β receptor type I (TGFβR1) using small molecules (SB431542 and LY2157299) to assess the effect of TGF-β inhibition on germ cell maintenance and differentiation *in vitro*. We used histological, gene expression and hormonal analyses to assess cellular organization, germ cell differentiation and androgen production.
Results: Our microscopic and histological observations showed reconstruction of seminiferous tubule-like structures within testicular organoids after treatment with TGFβR1 inhibitors. The organization of Sertoli, Leydig and germ cells was observed by immunostaining using specific markers (Vimentin, β-catenin, 3-βHsd, Ddx4, Pcna). Furthermore, TGFβR1 inhibitors promoted the proliferation of Sertoli and spermatogonia cells, as well as the differentiation of spermatogonia as demonstrated by upregulation of meiotic and post meiotic genes. The organoids treated with TGFβR1 inhibitors demonstrated significant increase in testosterone production as an indication of functional potential in comparison to the control group.
Conclusion: We have succeeded in reconstruction of seminiferous tubule -like structures from dissociated testicular cells through TGF-β inhibitors. This achievement may contribute to infertility treatment in the future by providing a more functional organoid model for drug screening and especially by suggesting SB431542 and LY2157299 as new therapeutic candidates.
Keywords: Spermatogenesis, Testicular Organoid, TGF-β Inhibitor, Tubule-Like Structures

Ps-49: Sub-Networks Identification in Brain Tumor Metastasized from Breast Cancer

Mousavi M*, Mir Rezayi SI, Eshkevari AM, Rezvani S, Saadati P, Bahremand MS, Akbari M, Estahri B, Shahsavari MH, Sahabjo A, Amini Najafi MA, Babayi S, Jalali SA, Sadri R, Tabatabaee MA, Salmani H, Nemati A, Salari A

Fakor High School, Qom, Iran
Email: asalari1365@gmail.com

Objective: Breast cancer metastases to the brain tissue. The tumor in new tissue is probably impacted from new environment. Our tries in this study are to dissected this type of change from molecular angles
Materials and Methods: The microarrays techniques are utilized with GEO2R software to revealed differentially expressed genes (DEGs) by p-value lesser or equal 0.05 and also Log-FC greater or equal 1 and lesser or equal -1 for Up and Down expressed genes respectively. which is a clue for molecular changes. After that, the functional analysis is performed by two KEGG and Gene Ontology databases to indicate each role of DEGs. In this way, the protein-protein interaction constructed by STRING database and sub-networks elicited via MCODE package by greater or equal 5 score.
Results: Authors reported 1160 DEGs, which are used in protein-protein interaction network (PPI) that showed 1010 nodes

and 6376 edges. In order, four sub-networks resulted from main PPI-Network which were higher or equal 5 score. Each sub-network analyzed by KEGG database and revealed some relation with brain tumors. The cluster1 indicate Protein digestion and absorption pathway and cluster2 report Cytokine-cytokine receptor interaction plus cluster3 and cluster4 indicates Th17 cell differentiation and Proteoglycans in cancer respectively.
Conclusion: In this study, four clusters reported in metastatic brain tumor from breast cancer. Each cluster has distinct role in breast cancer and migration particularly cluster4 by their role in metastatic process and adhesion function are candidates for therapeutic approaches in brain metastasized tumor from breast cancer.
Keywords: Breast Cancer, Personalized Cancer Medicine, PPIs, Systems Biology, Sub-Network

Ps-50: Potency of Alpha-Mangostin on The Proliferation of Human Umbilical Cord Mesenchymal Stem Cells

Nainggolan I[1, 2*], Manimpurung B[3], Novianti T[3]

1. Eijkman Research Center for Molecular Biology, National Research and Innovation Agency, Bogor, Indonesia
2. School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia
3. Department of Biotechnology, Faculty of Health, Esa Unggul University, Jakarta, Indonesia
Email: itam001@brin.go.id

Objective: Mesenchymal Stem Cells (MSCs) are adult stem cells that can self-renew and exhibit multilineage differentiation. The umbilical cord is an abundant sources of MSCs and can obtain it non-invasively. *In vitro* culture of human umbilical cord mesenchymal stem cells (HUC-MSCs) required growth factors to stimulate proliferation and differentiation. Result studies of several herbal active ingredients showed their potency in promoting the secretion of growth factors. Alpha (α)-Mangostin (α-MG) is the biologically active compound derived from the pericarp of Garcinia mangostana L., which shows various pharmacological properties. The study aimed to know whether we can use α-MG as growth in promoting proliferation stimulation.
Materials and Methods: We maintained the cell culture in an α-minimum essential media (MEM) complete medium until twice the passages. Next, we distributed an equal number of 1,6x104 cells with four repetitions into 96 well-plates. We carried out treatment of four α-MG dosages: 0.5 µg; 1 µg; 5 µg; and 10 µg in HUC-MSCs culture, with transforming growth factor-beta2 (TGF-b2) as a positive control and cells in a basic medium as a negative control. We incubated all the cell cultures at 370C, 5% CO_2 for 72 and 168 hours. We examined the efficacy of α-MG as a stimulant on the proliferation of HUC-MSCs using a WST-1 colorimetric assay and statistical package for social science (SPSS).
Results: Data analysis showed that the HUC-MSCs proliferation given α-MG treatment with positive control was significantly equal (sig. > 0.05) and had no significant difference. The α-MG treatment for 72 hours with concentrations of 5 µg/mL and 10 µg/mL is the best exposure time and concentration in increasing the proliferative ability of HUC-MSCs compared to the positive control.
Conclusion: The α-MG at certain levels and times of treatment has the potential to replace growth factors and support the proliferation of HU-MSCs cells.
Keywords: HUC-MSCs, Alpha-Mangostin, Proliferation, WST-1 Assay

Ps-51: Potency of Alpha-Mangostin in Supporting The Proliferation and Differentiation of Mouse Cortical Neural Stem Cells

Nainggolan I[1, 2*], Rusli F[3], Novianti T[3]

1. Eijkman Research Center for Molecular Biology, National Research and Innovation Agency, Bogor, Indonesia
2. School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia
3. Department of Biotechnology, Faculty of Health, Esa Unggul University, Jakarta, Indonesia
Emails: itam01@brin.go.id, titta@esaunggul.ac.id

Objective: Neural stem cells (NSCs) can differentiate into neurons, astrocytes, and oligodendrocytes and carry out self-renewal. Epidermal growth factor (EGF) and fibroblast growth factor (FGF-2) usually support the proliferation and differentiation of NSCs precursors. However, these growth factors are high prices. Therefore, we need alternative cheaper, more effective growth factors. α-Mangostin is one of the compounds extracted from the Garcinia mangostana L. Several studies reported that this herb contains compounds that can support stem cell cultures. This study aims to know the ability of α-Mangostin and its particular concentration to stimulate the growth and differentiation NCSs to nerve cells.
Materials and Methods: We maintained the NSCs culture in a NSCs' complete medium inside a flask coated with poly-L-ornithine and laminin. Then we incubated the cells at 37°C, 5% CO_2, for 72 hours with 5, 8, 10, and 15 µM concentrations of α-Mangostin. We examined the potency of α-Mangostin using a WST-1 colorimetric assay and quantitative real-time polymerase chain reaction (q-PCR) and analyzed it with statistical tests.
Results: Cell morphology analysis on α-Mangostin treated cells with concentrations of 5 µM and 8 µM can induce dendritic-like cell formation in P3-D1. The q-PCR analysis showed that Nestin and MAP-2 genes, markers of neuron cells, are expressed.
Conclusion: Our study showed that concentrations of 5 µM and 8 µM are suitable for stimulating the proliferation and differentiation ability of NSCs culture. Therefore, α-Mangostin has potency as a substitute for commercial growth factors such as FGF-2 and EGF.
Keywords: Alpha-Mangostin, NCSs, Nestin, MAP-2

Ps-52: Anti-Inflammatory Properties of Pregnenolone after Induction of Inflammation by Lipopolysaccharide (LPS)

Negintaji K[1*], Ghanbari A[1], Frozanfar M[2], Jafarinia M[2]

1. Cellular and Molecular Research Center, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran
2. Department of Biology, Marvdasht Branch, Islamic Azad University, Cellular and Molecular, Marvdasht, Iran
Email: amirghanbari52@yahoo.com

Objective: Neurosteroid pregnenolone can act as an anti-inflammatory molecule. In neuroinflammatory diseases, a decrease in the level of pregnenolone has been observed, which can emphasize the neuroprotective and anti-inflammatory role of this neurosteroid. Therefore, the aim of this study was to investigate the role of neurosteroid pregnenolone in the inflam-

matory culture medium containing mouse neural stem cells (NSCs) and its effect on inflammatory and oxidant factors.
Materials and Methods: In this experimental study, NSCs were prepared from the ganglionic eminences of the brain of E14- mouse embryos. The cell viability was done by MTT method and two ways of treatment with the pregnenolone alone and co treatment with LPS in the culture medium. The number of neurospheres and cells derived from neurospheres were counted. Then the supernatant of the cells was removed and the levels of inflammatory markers IL6 and TNFα, oxidant and antioxidant markers (MDA, NO and FRAP) were measured by ELISA method. The data were analyzed by one-way variance statistical method and Tukey post-hoc test.
Results: LPS caused a significant decrease in the survival of NSCs compared to the control group. In the co treatment with LPS and pregnenolone and pregnenolone alone , the number of neurospheres and cells derived from it increased significantly compared to the LPS group. Pregnenolone alone and with LPS significantly reduced IL-6 and TNF-α levels compared to the LPS group. On the other hand, pregnenolone significantly reduced level of NO and MDA from inflammation by lipopolysaccharide, in addition, a significant decrease in the amount of NO and MDA were observed in the co treatment of pregnenolone and LPS in different concentrations compared to the LPS group.Conclusion In the co treatment of LPS and pregnenolone and pregnenolone alone the viability of stem cells and neurospheres and cells derived from it increased significantly compared to the LPS group. Pregnenolone alone and with LPS significantly reduced the levels of IL-6 and TNF-α and the levels of NO and MDA induced by lipopolysaccharide inflammation.
Conclusion: In the co treatment of LPS and pregnenolone and pregnenolone alone, the viability of stem cells and neurospheres and cells derived from it increased significantly compared to the LPS group. Pregnenolone alone and with LPS significantly reduced the levels of IL-6 and TNF-α and the levels of NO and MDA induced by lipopolysaccharide inflammation.
Keywords: Inflammatory Factors, Lipopolysaccharide (LPS), Mouse Neural Stem Cells, Pregnenolone, Oxidant Factors

Ps-53: Fabrication and Evaluation of Resveratrol Coated Electrospun Chitosan/ Polyethylenoxide Nanofibers

Nejaddehbashi F*, Moghadam A, Orazizadeh M

Cellular and Molecular Research Center, Medical Basic Sciences Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Email: fereshte_negad@yahoo.com

Objective: Wound healing is a complicated cellular process involving various mechanisms getting it intricate for designing a scaffold for therapeutic applications. This study deals with the designing and development of electrospun chitosan-polyethylene oxide nanofiber wound dressing for capable simultaneous drug delivery. Resveratrol were chosen for coating on chitosan based nanofibers for their efficacy in pro-angiogenic and wound healing activities.
Materials and Methods: In this experimental study, an electrospun nanofibrous mat composed of CS/PEO and subsequent coating by 0.05 and 0.1% Resveratrol was prepared. Structural, mechanical, and biological properties of nanofibers were evaluated using SEM, tensile testing, *in vitro* release study and MTT assay was done.
Results: According to *in vitro* results, cells adhered and proliferated in two scaffolds. However, cells deep into the scaffold were more detected in the CS/peo 0.1% resveratrol. Angiogenesis was seen in 0.05% resveratrol CS/peo.
Conclusion: The electrospun CS/peo/RS 0.1% could be considered as a promising type of wound dressing in wound healing process *in vivo*.
Keywords: Chitosan, Electrospinning, Polyethylene Oxide Healing, Resveratrol, Wound Healing

Ps-54: Alginate Containing Umbilical Cord Wharton's jelly Can Promote Wound Healing in Rat

Nejaddehbashi F*, Azarbarz N, Shafiee Z, Bijannejad D, Khorsandi L

Ahvaz Jundishapur University of Medical Sciences, Cellular and Molecular Research Center, Medical Basic Sciences Institute, Ahvaz, Iran
Email: fereshte_negad@yahoo.com

Objective: The first line of defense against the environment is the skin. Regular wound dressings are insufficient on wound healing because of their poor prognosis, nature, and excessive discharge. The main purpose of this study is to encapsulate Wharton's jelly pieces in to an alginate scaffold to repair skin tissue.
Materials and Methods: In the first step an Alginate /Wharton's jelly scaffold was fabricated with simple method. To enhance Wharton's jelly's wound healing abilities through the release of growth factors like EGF and bFGF, tiny pieces of the jelly were encapsulated in an alginate scaffold. The designed scaffold was evaluated by SEM, tensile test, swelling ratio test and growth factor release evaluation .The scaffolds' cytotoxicity toward human Wharton's jelly's cells was examined using the indirect MTT test. *In vitro* wound closure was evaluated using a scratch test. Alginate scaffolds containing Wharton's jelly pieces are biocompatible and have higher cell viability, according to MTT data.
Results: We found that Wharton's jelly increases biocompatibility and wettability of scaffold, cell proliferation, *in vitro* and Wound healing Potential of designed scaffold was evaluated *in vivo*.
Conclusion: Overall, the results revealed the better recovery for rats treated with our scaffold. This combination is suggested for use in skin tissue engineering and wound healing.
Keywords: Growth factors, Hydrogel Scaffold, Mesenchymal Stem Cells, Wound Healing, Wharton Jelly

Ps-55: Effects of Ethanol and Mitomycin on Survival of Rat Limbal Epithelial Stem Cells: An *In Vitro* Study

Nejaddehbashi F*, Nasrolahi A, Bayati V, Zamani M

Cellular and Molecular Research Center, Medical Basic Sciences Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Email: fereshte_negad@yahoo.com

Objective: Ethanol and mitomycin C (MMC) are clinically used for the treatment of corneal diseases such as LASEK and LASIK surgery. In this study we investigated the effects of time dependent alcohol and MMC in *in vitro* cultured rat limbal stem cells (LESCs) to determine the appropriate time for use of this compound in the clinic.

Materials and Methods: LESCs (n=10 eyes) isolated from male Wistar rats were cultured, characterized and divided into 3 groups. One group were exposed to 20% concentration of ethanol for 5, 10, 15, 20, 25, 30 seconds and cell viability were assessed for 1, 3 and 5 days after ethanol exposure using MTT assay. To investigate the effect of MMC, second cell group were treated with 0.02% MMC in various periods (15s, 30s, 60s, 90s, 120s) and time-dependent responses of cultured LESCs were recorded. Third cell group co-treated with ethanol and MMC and then dose and time dependency were evaluated.
Results: In comparison with the viable cells in the control group, ethanol markedly decreased the viability of cells in a time dependent manner in day 1 and 3. In day 5, the viability of LESCs improved significantly ($P<0.05$) in comparison with day 1. The numbers of viable stem cells were significantly decreased by MMC treatment in a time-dependent manner as determined by MTT assay ($P<0.001$). The use of mitomycin along with alcohol decreased in cell viability in all treated groups with ethanol+MMC in comparison with control in day 1, 3 and 5 ($P<0.0001$).
Conclusion: Our findings suggest that ethanol and MMC reduced cell viability in cultured LESCs in a time dependent manner and have no long-time effects.
Keywords: Corneal Epithelium, Ethanol, Ocular Surgery, Limbal Stem Cells, Mitomycin C

Ps-56: Designing A Complex to Activitie LRRK2 Gene in Parkinson with VP64-Based CRISPRa Meditated Gene Activation

Nematzadeh F*, Sadeh sani MM

Esmat High School, Shut, Iran
Email: fereshteh.nematzadeh@gmail.com

Objective: Designing a Complex to Activitie LRRK2 Gene in Parkinson with VP64-based CRISPRa meditated Gene Activation Fereshteh Nematzadeh1, Mohammad Mehdi Sadehsani2 1-Esmat High school 2- Department of Supreme Association of Iranian Abstract: Parkinson's disease (PD), is a progressive, destructive and long-term disorder of the central nervous system that mainly disrupts the movement system of the body. The symptoms of this disease usually appear slowly and gradually, and with the progress of the disease, immobile symptoms also appear. The most obvious early signs of this disease are tremors, dryness of the body, relaxation of movements and difficulty in walking. The cognitive and behavioral symptoms of this disease usually appear in most people in the form of depression, anxiety and lack of interest and excitement. In the advanced stages of Parkinson's disease, sometimes dementia is also common. A person with Parkinson's may also experience problems with sleeping and their sensory system. The motor symptoms of this disease occur due to the loss of cells in the substantia nigra of the brain, resulting in a decrease in dopamine (which is a neurotransmitter). Dopamine is important for maintaining the body's natural movement patterns, and this is exactly why many Parkinson's treatments are aimed at increasing dopamine levels in the brain. In Parkinson's disease, the loss of neurons (nerve cells) also occurs in other parts of the brain and becomes the basis for some non-motor symptoms of this disease. CRISPR is a new type of genetic editing technology that can greatly contribute to the development of gene therapy. Until now, gene therapy has been mainly done through the "gene transfer" technique; In this way, a harmless virus transfers a healthy copy of a gene to the cell to take the place of the defective gene that caused the disease. But in the CRISPR method, scientists can directly correct the faulty gene. They remove the defective DNA and replace it with healthy DNA. Basically, this method should work much better than adding a new gene, because then the risks of adding a foreign gene are eliminated.
Materials and Methods: All gRNAs were designed to activate X gene by Chop-Chop, and after quality control and verification of their qualifications, the first five that reported the highest level of specificity and the lowest level of off-targets were selected. Then, with the help of off-targets checking tools, their position was analyzed.
Results: According to bioinformatics studies conducted on large gene families, related genes Specific with Parkinson's disease and include: PARKIN/ PINK1/ DJ1/ LRRK2/ ANCA/ NCA2/ NR4A2/ UCHL1 and some other genes. Among the identified genes, LRRK2/SNCA genes with autosomal dominant inheritance pattern and PINK1/PAK7/PARK genes with autosomal recessive inheritance pattern undergo changes that cause the disease and are inherited from the patient's parent.
Conclusion: Although these studies have significantly contributed to the development of CRISPRa technology, most of them have been conducted in *in vitro* and *ex vivo* cell culture systems, and there are still challenges regarding *in vivo* applications. Specifically, several studies have reported on the *in vivo* toxicity of CRISPRa components. For example, it has been reported that VPR and SAM are toxic when highly expressed in Drosophila with a strong promoter. Also, in mice, ubiquitous expression of VPR during development and expression in inhibitory neurons are toxic. On the other hand, ubiquitous expression of Cas9 18 and dCas9-VP64 6 in mice is not overtly toxic, suggesting that neither dCas9 nor VP64 is toxic *in vivo*, and that high expression of either p65 or Rta, or both, may be responsible for the observed toxicity. Therefore, it is crucial to develop CRISPRa technologies that take into account both the efficiency of gene activation and side effects for *in vivo* applications. Based on these findings, we hypothesized that a simple VP64-based CRISPRa would be useful *in vivo* in a variety of cell types, developmental stages, and pathological conditions. However, although there have been comparative studies of next-generation CRISPRa constructs such as SAM, VPR, and SPH with high transcriptional activation capacity, to the best of our knowledge there are not enough studies that seek to improve and characterize VP64-based CRISPRa by directly comparing activities in a systematic, controlled fashion. Here, we aimed to characterize and rationally design approaches for VP64-based transcriptional activation of both endogenous and exogenous genes.
Keywords: CRISPR, LRRK2, Parkinson, VP64

Ps-57: The Synergistic Effects of Homogenized Coumarin and Bacterial Nanocellulose on Skin Wound Healing in A Rat Model

Noori M*, Cheraghi H, Ghashghaii A

Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
Email: dr.hadi.cheraghi@gmail.com

Objective: Wound healing is a complex and orchestrated biological process involving coagulation, inflammation, tissue regeneration, and structural remodeling to restore damaged tissues and promote wound closure. The study investigated the

therapeutic potential of combining coumarin and bacterial nanocellulose to enhance skin wound healing.
Materials and Methods: Full-thickness excisional wounds were induced on the dorsal area of female adult Sprague Dawley rats. The rats were divided into five groups: a control group treated with sterile gauze, a group treated with basal cream ointment, a group treated with 2% coumarin cream, a group treated with a combination of 2% coumarin and nanocellulose, and a group treated with nanocellulose alone.
Results: Wound area was assessed regularly, and histological analyses were conducted on post-wound days 7, 14, and 21. The results of this study demonstrated noteworthy advancements in various wound healing parameters, including wound closure rates, hydroxyproline content, collagen deposition, and histopathological features, particularly on day 7, within the coumarin and coumarin + nanocellulose treatment groups, when compared to both the control group and other treatment groups. Moreover, the evaluation of the Oxidative Stress Index, which serves as an indicator of inflammation and oxidative stress, revealed a significant improvement in the coumarin and coumarin + nanocellulose groups.
Conclusion: This study illuminates the tremendous potential of homogenized coumarin, both as a standalone treatment and in combination with bacterial nanocellulose, to significantly enhance skin wound healing in a rat model. The observed improvements in wound closure, collagen synthesis, and reduced oxidative stress suggest that coumarin and coumarin + nanocellulose interventions hold great promise as captivating therapeutic approaches for promoting wound healing not only in preclinical models but also in animals and humans suffering from various types of wounds. Further research endeavors are warranted to unravel the underlying cellular and molecular mechanisms of these therapeutic effects, while also conducting rigorous evaluations of their safety and efficacy in both animal and human trials.
Keywords: Collagen, Coumarin, Nanocellulose, Oxidative Stress, Wound Healing

Ps-58: Fibrotic Liver ECM-Derived Microparticles Enhanced Cancerous Phenotype of HCC Cells in Biomimetic Liver Microtissues

Nouri K[1], Seydi H[1], Piryaei A[2], Vosough M[1, 3*]

1. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Research Center for Biochemistry and Nutrition in Metabolic Diseases, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran
3. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Stockholm, Sweden
Email: masvos@Royaninstitute.org

Objective: Hepatocellular carcinoma (HCC) is the fourth most fatal cancer around the world which often initiates after chronic liver fibrosis and cirrhosis. During liver fibrosis extracellular matrix (ECM) undergo chemical and physical changes which might contribute to development of cancerous phenotype. However, the effect of fibrotic ECM on HCC deterioration is not properly understood. This study aims to identify whether alteration in biochemical compounds of fibrotic ECM can regulate the HCC cells' characteristics.
Materials and Methods: In order to produce a liver model including ECM, we co-cultured Huh-7 cells, human umbilical vein endothelial cells (HUVECs) and LX-2 cells in the presence of cell-sized microparticles (MPs) which is derived from decellularized tissue from CCl4-treated fibrotic (fibrotic microtissues) or normal (normal microtissues) livers. The liver microtissues were cultured *ex vivo* for 14 days, then compared in aspect of gene expression.
Results: The results indicated that the EMT-related gene, MMP9 increased and CDH1 decreased in fibrotic microtissues compared to normal ones.
Conclusion: In conclusion, fibrotic ECM enhanced mesenchymal phenotype in liver microtissues.
Keywords: Epithelial to Mesenchymal Transition (EMT), Extracellular Matrix, Hepatocellular Carcinoma, Liver Fibrosis, Liver Micro-Tissue

Ps-59: Curcumin Reduces the Viability and Proliferation in the OVCAR-3 Cell Line of Human Ovarian Cancer

Panahi Alanagh S*, Talkhabi M

Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
Email: m_talkhabi@sbu.ac.ir

Objective: Ovarian carcinoma is a deadly disease with a low cure rate due to rapidly growing tumors. Ovarian cancer cells mainly spread within the peritoneal cavity. Curcumin, which is derived from the rhizome of the Curcuma longa plant, has antioxidant, anti-inflammatory, and anti-cancer properties. Recent research suggests that the ability of curcumin to prevent cancer may be attributed to its capacity to hinder cell growth and viability.
Materials and Methods: In this study, cells (1×10^4/well) were seeded in 96-well plates. After 48 hours, the cells were treated with various concentrations of curcumin, ranging from 0 to 100 µM. The MTT assay was performed after 24 hours of treatment to evaluate cell viability.
Results: Our data revealed that curcumin could significantly inhibit growth and viability in OVCAR-3 cells. Cell viability after 24 hours of treatment with concentrations of 10, 20, 30, 60, 70, and 80 µM shows a significant difference ($P<0.001$), which is a sign of dose dependence. The cells exposed to 30 µM curcumin exhibited obvious changes in morphologic characteristics. There were more shrinking cells and floating cells that had lost their inhibiting ability in the curcumin-treated group than in the controls.
Conclusion: According to the results, which include part of our research, it is thought that curcumin may have a positive effect on the treatment of ovarian cancer. Our research will continue.
Keywords: Curcumin, MTT, Ovarian Cancer, OVCAR-3, Tumor

Ps-60: Optimizing A Electrospun Hybrid Naostructure from Ttype 1 Collagen, Polyvinyl Alcohol and Propolis for Tissue Engineering Application

Parham A[1, 2*], Mehrvarz T[1], Kaka Gh[3], Mahdavi H[4]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Mashhad, Iran
2. Stem Cell Biology and Regenerative Medicine Research Group, Research Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

3. Department of Anatomy, Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Department of Novel Drug Delivery Systems, Faculty of Science, Iran Polymer and Petrochemical Institute, Tehran, Iran
Email: parham@um.ac.ir

Objective: In recent years, there is a growing interest in the creation of biocompatible nanostructures for tissue engineering, which are classified into polymer, carbon, mineral, and composite kinds based on the component materials and manufacture techniques.
Materials and Methods: In this study, we tried to optimize the method of creating a biocompatible hybrid nanostructure composed of type 1 collagen and polyvinyl alcohol polymer (PVA) with an alcoholic extract of propolis. Polyvinyl alcohol is a biocompatible and non-conductive polymer; Type 1 collagen is extracted from mouse tails as a natural ECM material, and bee propolis is known for its natural anti-inflammatory and antimicrobial properties. The nanostructure was created using an electrospinning machine. Based on the assessments during the nanostructure creation process, it was determined that the most effective parameter in collagen electrospinning is solvent type, solvent concentration and feeding rate.
Results: Here, phosphate-buffered saline (PBS) and 70% ethanol were used as collagen solvents, while PVA was dissolved in deionized water. For propolis, its flavonoids were measured and a suitable method without heating was selected for extraction. At the end, the physical properties of the produced nanostructures were examined using SEM and spectrometry.The results showed that the diameter of hybrid nanofibers is affected by the type and concentration of the solvent, as well as the solution's viscosity.
Conclusion: In conclusion, setting the optimized parameters result in the formation of the best matrix with small fibers and high porosity for tissue engineering purposes.
Keywords: Collagen, Nanofibers, Polyvinyl Alcohol, Propolis, Tissue Engineering

Ps-61: Gallic Acid Prevented Oxidative Stress Induced by Di-2-Ethylhexyl Phthalate to Improves Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells Through Activation of BMP/RUNX2 Pathway

Parvaz M*, Abnosi MH

Department of Biology, Faculty of Basic Sciences, Arak, Iran
Email: Mahrokhm129@gmail.com

Objective: di-2-ethylhexyl phthalate (DEHP) is a plasticizer, used in preparation of polyvinyl chloride products from food-container to medical-devices. Bone marrow mesenchymal stem cells (BMSCs) as osteoblast-precursors are exposed to DEHP which hinders its differentiation. Since gallic acid (GA) improves osteogenic differentiation of BMSCs, in this study it was used to prevent toxic effect of DEHP.
Materials and Methods: BMSCs were extracted from Wistar rats and treated with 100 µM of DEHP and 0.25 µm GA individually and in combination for 20 days. Further analysis includes matrix production, total protein content, alkaline-phosphatase activity, MDA level, total antioxidant capacity, CAT and SOD activity and osteogenic related gene expression were carried out.
Results: Significant decrease in bone matrix production was observed in DEHP treated cell whereas GA prevented the hindering effect of this chemical. In addition, significant increase of BMP2, RUNX2 and ALP genes expression was detected which was imprinted in ALP activity elevation. DEHP induced oxidative stress which was ameliorated by GA through reduction of MDA production and improvement in activity of CAT and SOD.
Conclusion: GA was able to compensate the toxic effect of DEHP, and improved osteogenic differentiation of BMSCs through BMP/RUNX2 pathway.
Keywords: BMSCs, DEHP, Galic Acid, Oxidative Stress, Osteogenic Differentiation

Ps-62: The Establishment of An *In Vitro* Cardiac Fibrosis Model for Mechanistic Studies

Pourmehran Y[1, 2*], Pahlavan S[1], Vahdat S[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 Developmental Biology, University of Science and Culture, Tehran, Iran
3. Applied Cell Sciences Division, Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: sarah.pahlevan2007@gmail.com

Objective: Heart injuries from multiple causes can result in pathological remodeling and fibrosis, which in turn promote the development of heart failure. An acute myocardial infarction leads to an increased proliferation of cardiac fibroblasts (CFs) and the transition to the myofibroblast phenotype. During this fibrotic phase, myofibroblasts begin to secrete elevated levels of collagens and other ECM proteins. Moreover, fibrogenic growth factors such as transforming growth factor-β, cytokines including tumor necrosis factor-α, interleukin (IL)-1 and IL-6, and neurohumoral pathways trigger fibrogenic signalling cascades, which further promote fibrosis. In this study, we aimed to establish an *in vitro* cardiac fibrosis model to provide a platform for mechanistic studies at transcriptome and proteome level.
Materials and Methods: Human CFs were isolated from myocardial biopsies obtained during mitral valve replacement. The biopsies were cut into 1 mm2 pieces and explanted on the gelatin-coated plates. Explants were cultured overnight in FBS and thereafter, for one week in fibroblast culture media. Fibrosis induction was performed by employing three concentrations of Doxorubicin (DOX; 0.1, 0.25 and 0.5 µM) for 72 hours, and it was assessed by immune-staining of smooth muscle actin (α-SMA) and analysis of secreted collagen content.
Results: Isolated fibroblasts expressed CF-specific proteins; Vimentin, Collagen a1 and CD90. Treatment with 0.1 and 0.25 µM of DOX did not significantly affect cell viability or cell apoptosis, whereas it provoked fibroblasts activation. Myofibroblast induction was confirmed by staining against α-SMA, which is a fibrosis-related biomarker. Furthermore, DOX-treated cells secreted more collagen into the culture medium.
Conclusion: Our results showed that 0.1 and 0.25 µM concentrations of DOX can activate CFs and promote their transdifferentiation into myofibroblasts. This *in vitro* model of cardiac fibrosis can be served as a platform to study the underlying mechanisms of cardiac fibrosis, to evaluate the fibrosis-related transcriptional changes, and to develop novel therapeutic strategies.
Keywords: Cardiac Fibrosis, Cardiac Fibroblast, Disease Modeling, Doxorubicin, Myofibroblasts

Ps-63: The Effect of Autologous Serum on The Growth of Menstrual Blood Mesenchymal Stem Cells

Rezaei Kiasari Z[1*], Zare F[1], Khodashenas Sh[2]

1. Department of Medical Biotechnology, Mazandara University of Medical Sciences, Sari, Iran
2. Department of Medical Biotechnology, Mazandara University of Medical Sciences, Sari, Iran
Email: s.khodashenas@mazums.ac.ir

Objective: Menstrual blood-derived mesenchymal stem cells (MB-MSCs) were recently defined and express CD44, CD90 and CD105 markers. One of the advantages of these cells is easy collection and lack of ethical issues. Therefore, Men-MSCs can be considered as a good source for research and regenerative medicine. Cell culture is a technique in which cells are grown under controlled laboratory conditions. Serum is an important part of the culture medium. Serum is a protective solution containing growth factors and hormones. The most common serum in cell culture is fetal bovine serum (FBS). FBS is an unspecified mixture and may contain endotoxin or prion proteins. Therefore, human alternative FBS is needed for clinical applications. Here, a human protocol is tested, using autologous serum (AS) instead of FBS to grow MSCs derived from the individual's own MB.
Materials and Methods: MB was collected on the second day of menstruation and a blood sample was taken from the person to prepare AS. MSCs were isolated from MB and cultured in culture medium enriched with AS with concentrations of 10, 15 and 20%. Then growth and proliferation and surface markers were measured.
Results: Cells grew and proliferated in all three concentrations of AS, but optimal growth and proliferation was observed at 15% concentration. Also, cultured cells expressed mesenchymal markers.
Conclusion: As a result, since the use of MSCs is increasing day to day and the safety of the process and materials used in regenerative medicine is crucial, AS can be considered a suitable option for the growth and proliferation of MB-MSCs.
Keywords: Autologous Serum, Menstrual Blood Mesenchymal Stem Cells, Fetal Bovine Serum,

Ps-64: Generating Expandable Cardiac Progenitor Cells from Human Pluripotent Stem Cells

Rezaeiani S[*], Pahlavan S , 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: Baharvand@RoyanInstitute.org

Objective: Cardiovascular diseases (CVDs) are a significant cause of mortality worldwide. Although pharmacological and surgical interventions have improved patients outcomes, they do not address the underlying cause of the disease or fully restore heart function. Recently, there has been increasing interest in using cardiac progenitor cells (CPCs) derived from human pluripotent stem cells (hPSCs) to regenerate damaged heart tissue. CPCs can differentiate into cardiomyocytes, endothelial, and smooth muscle cells, making them an attractive therapeutic option for CVDs. However, the lack of a standardized protocol for generating expandable CPCs has limited their research and clinical application. In this study, we aimed to develop an efficient protocol for producing expandable CPCs from hPSCs and differentiating them into the three main cell lineages of the heart.
Materials and Methods: We expanded hPSCs in a 3D culture and induced them into CPCs using a chemically defined small molecule (SM)-based protocol as follow: CHIR for one day, followed by a medium without the small molecule for another day. Thereafter, differentiating cells were treated with IWP2, SB431542, and Purmorphamine for two days. The resulting spheroids were dissociated into single cells and cultured on Matrigel-coated plates with specific factors including 0.5 μM A83-01, 100 ng/ml bFGF, and 3 μM CHIR, for up to 4 passages. We assayed the potential of passage 4 expandable hPSC-derived CPCs for differentiation into the three main cardiac cell types, by specific growth factor treatments for each lineage over 12 days.
Results: Flow cytometry analysis revealed that on the fourth day of differentiation, 77 ± 5% of hPSC-derived CPCs expressed the NKX2.5 marker. After 4 passages, expandable CPCs could differentiate into cTNT+ cardiomyocytes, VWF+ endothelial cells and SMA+ smooth muscle cells with high efficiency
Conclusion: In conclusion, this protocol might enable the generation of large scale hPSC-derived CPCs for research and clinical application.
Keywords: Cardiovascular Diseases, Cardiac Progenitor Cells, Differentiation, Regenerative Medicine

Ps-65: Effect of Mineral-Loaded Acellular Ovine Small Intestine Sub Mucosa on Burn Models

Roshangar L[1*], Yaghoobzade A[2], Khanzadeh A[2], Nikzad S[2]

1. Department of Anatomical Science, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Paramedical science, Tabriz Azad University of Medical Sciences , Tabriz , Iran
3. Department of Biological and Medical, Montreal, Canada
Email: Lroshangar@yahoo.com

Objective: Injury from the severe burn is exacerbated by a persistent inflammatory response. This response is mediated by cytokines and chemokines, which are released from various immune cells, including mast cells.
Materials and Methods: In this study, the ability of the acellular ovine small intestine submucosa (AOSIS) to load and release of minerals was first investigated, and it was found that the preparation of the scaffold by a modified method enables it to load and release water-soluble drugs. Then, 28 male Wistar rats were divided into four groups, a third-degree burn was created, and except for the control group, the others were treated with: AOSIS, WJ-MSCs seeded AOSIS, or AOSIS loaded with WJ-MSCs and Minerals.
Results: Wound sampling on the 5th day after treatment showed that the number of intact and degranulated mast cells in the treatment groups was associated with a decrease compared to the control group. In the last group, this decrease was the largest [and statically significant ($P<0.05$)].
Conclusion: Our results indicate that AOSIS loaded with WJ-MSCs and MP could be used as an innovative tissue-engineered device to control inflammatory condition during burn wound healing.
Keywords: Mesenchymal Stem Cells, Scaffold, Tissue Engineer-

ing, Wound Healing

Ps-66: Investigating The Effect of Mesenchymal Stem Cells-Conditioned Media and Exopolysaccharide Combination on The Migration and Proliferation of Fibroblast and Endothelial Cells in High Glucose Culture Medium as An *In Vitro* Diabetic Model

Rostami H[1*], Brouki Milan P[2], Vousooghi N[1], Haramshahi SMA[2], Verdi J[1]

1. Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: Jverdi49@gmail.com

Objective: Proliferation and migration of fibroblast and endothelial cells play an important role in wound healing. The therapeutic effect of mesenchymal stem cells-conditioned media(MSCs-CM) on skin wound healing have before been investigated. Also, the effect of exopolysaccharides on angiogenesis and wound healing has been reported. The impact of exopolysaccharide produced by cold-adapted yeast R. mucilaginosa sp. GUMS16 (GUMS16 EPS) on wound healing has been evaluated, recently. However, the effect of GUMS16 EPS on the behavior of fibroblast and endothelial cells has not been investigated in previous studies. So, in this study, we explored the impact of Wharton's jelly-derived MSCs-CM (WJ-MSCs-CM), GUMS16 EPS and their combination on proliferation and migration of human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs).
Materials and Methods: MTT assay and scratch assay was used to measure cell proliferation and cell migration, respectively. The cells were cultured in a high-glucose medium as an *in vitro* diabetic model.
Results: Our finding showed that treatment of HDFs with WJ-MSCs-CM and GUMS16 EPS resulted in increased cell proliferation. While WJ-MSCs-CM had not significant impact on HUVECs proliferation, GUMS16 EPS led to an increase proliferation. The treatment of cells with WJ-MSCs-CM led to an increase in cell migration, but GUMS16 EPS did not significant effect on the cell migration, while the treatment of cells with the combination of WJ-MSCs-CM/GUMS16 EPS increased the cell migration rate more than WJ-MSCs-CM.
Conclusion: These results suggest that WJ-MSCs-CM/GUMS16 EPS combination treatment of diabetic wound may be a feasible strategy for wound healing.
Keywords: Conditioned Media, Exopolysaccharides, Mesenchymal Stem Cells, Wound Healing

Ps-67: Ascorbic Acid and Fibroblast Growth Factor-2 Promotes Cardiomyocyte Differentiation from Stromal Vascular Fraction

Sabet zadeh N[1, 2*], Mokhber M, Najd Ghahremani A[3], Jabbari Fakhr M[4], Sadeghian Chaleshtori S[3]

1. Institute of Biomedical Research, University of Tehran, Tehran, Iran
2. Doctorate of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Department of Tissue Engineering, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran
Email: neda.sabetzadeh@gmail.com

Objective: Cardiovascular diseases are one of the main causes of death in the world. Because the heart has a weak internal repair mechanism, the replacement of lost cells is one of the important goals of regenerative medicine. Stem cells are a renewable resource for repairing cardiac lesions and among this adult cell sources, such as adipose-derived stromal vascular fraction (SVF) is very important because of non-Invasive access. In Novel strategies growth factors have been investigated on cell treatment for effective cardiac tissue recovery. The aim of this study was to culture mesenchymal stem cell derived from sheep adipose tissue and differentiate them into cardiomyocyte-like cells and surgically transplant them into infarcted heart in sheep.
Materials and Methods: SVF cells were isolated from sheep adipose tissue. differentiation steps were including reduction of FBS from 15 to 5% and for 4 hours. 0% for cell synching, using azacitidine solution as the primary differentiation factor with two concentrations 5 and 10 μM, using the differentiation culture medium for cardiomyocyte-like cells containing FGF and vitamin C for 21 days. Morphological structures of cell development were detected. Cardio myocyte phenotypes were characterized with cardiac specific genes expression.
Results: After 21 days, elongated myotubule cells, transvers connection, star-shaped were observed. Their growth rate also decreased significantly. The cells verified via GATA-4, expression factor, cardiac troponin I, myocyte enhancer factor 2c.
Conclusion: Overall these results suggest that an effective minimally invasive for isolation and co-treatment with FGF-2 and vit c would be beneficial in obtaining cardiomyocyte like cell and could be open up new possibilities in cardiac cell therapies
Keywords: Ascorbic Acid, Cardiomyocyte, FGF-2, SVF

Ps-68: A Novel Thermo-Sensitive and Conductive Hydrogel for Cardiac Application

Sabet zadeh N[1*], Barzegar F[1], Mokhber M[2], Jabbari Fakhr M[3], Sadeghian Chaleshtori S[4]

1. Doctorate of Veterinary Medicine, Faculty of Veterinary Medicine, Tehran, Iran
2. Institute of Biomedical Research, University of Tehran, Tehran, Iran
3. Department of Tissue Engineering, Faculty of Medicine Qom University of Medical Sciences, Qom, Iran
4. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: neda.sabetzadeh@gmail.com

Objective: Cardiovascular diseases are the first cause of death in Iran. cardiomyocytes have a limited regenerative capacity. Therefore, the use of tissue engineering to design a 3D structure outside the body can be an effective treatment for the renewal of the damaged myocardium. Novel treatment strategies designed at achieving long-term functional stabilization and improvement in heart function post MI include the delivery of biomaterial hydrogels and myocardial matrix-based therapies. Injectable hydrogels are as a minimally invasive therapeutic methodology. Hydrogels not only allow cells to connect, but

also causes cell migration, transfer of biochemical factors, diffusion of nutrients, waste materials. Injectable thermosensitive hydrogel scaffold is a three-dimensional polymer plexus insoluble in water, which has the ability to absorb body fluids in biological environment. Mechanical properties, biological properties and volume increase are among the most important characteristics of hydrogels.
Materials and Methods: The hydrogel formulation was based on aquase solution polyvinyl alcohol 10 %, acidic solution of chitosan 2 % and 50 % beta glycerol phosphate as a cross linker. Then gellation behavior, biodegradability, biocampatibility, sterility of the novel gel was examined in this research.
Results: The result indicates that encapsulated cell exhibits good viability in hydrogel via alamarblue assay. Suitable gelation time specially for using in surgery abaot 10 min, degradation rate and low cytotoxicity and good conductivity display potential for therapeutic use of cardiac repair.
Conclusion: In conclusion we found that this hydrogel was an appropriate candidate for MI treatment and drug or cell delivery activity in cardiac disorder.
Keywords: Hydrogel Scaffold, Cardiac, MI

Ps-69: Novel Hub genes Identified as Key Players in Macrophage Cells in Multiple Sclerosis Diseases

Salari A*, Jalali SA, Estahri B, Babayi S, Saadati P, Tabatabaee MA

Fakor School, Qom, Iran
Email: asalari1365@gmail.com

Objective: A molecular investigation reports several actors from inside macrophage cells, which are usually used for therapeutic approaches. To this end, this study is an effort to identify suitable players for cellular therapeutics in multiple sclerosis (MS) diseases.
Materials and Methods: The authors used bulk RNA-seq techniques to investigate differentially expressed genes (DEGs) in 138 MS patients via the DESeq2 package. After that, to reveal which functions are responsible for up and down genes, the biological process database was used. In order, the Cytoscape software has been used to identify protein-protein interactions between up-and down-regulated genes using a string database. Finally, the hub genes have been revealed by the CentiScape package.
Results: In this study, respectively, 132 and 296 up- and down-expressed genes are reported, which revealed some roles in the Regulation of Sodium Ion Transmembrane Transporter Activity for up-expressed genes and also Regulation Of Blood-Brain Barrier Permeability in down-expressed genes, which means the brain might be under attack. In addition, the PPIs network was constructed to evaluate the interaction between up and down genes, so both types of genes merged together, and the only 86 proteins recognized in the STRING database So, the PPIs network resulted in 86 nodes and 166 edges from up- and down expressed genes. In order, as hub genes, down-expressed CTLA4 and up-expressed IL13, and then down-expressed CCL4 were reported by 17, 13, and 13 interactions, respectively.
Conclusion: In this study, three hub genes and two signaling pathways were reported as the main actors in macrophage cells in MS patients. These molecular investigations could improve our knowledge of MS disease, in particular macrophage cells.
Keywords: Hub Genes, Multiple Sclerosis, PPIs, Systems Biology

Ps-70: Identification Novel Sub-Networks in Macrophage from Multiple Sclerosis

Salari A*, Akbari M, Mousavi SMM , Bahremand MS, Sahabjo A, Rezvani S, Shahsavari MS

Fakor School, Qom, Iran
Email: asalari1365@gmail.com

Objective: Multiple sclerosis, as a problem, is without any certain solution. This condition is associated with our knowledge of molecular behavior in consisting cells. To this end, we try in this study to understand more about molecular behavior through the protein-protein interaction network.
Materials and Methods: We used the RNA-seq dataset GSE137143 from GEO to investigate differentially expressed genes (DEGs) from 138 MS patients in macrophage cells via R packages such as DESeq2. In addition, to construct the PPI network, we utilize the STRING database from Cytoscape software through the merged up- and down expression genes. Furthermore, to elicit sub-networks, the author used the MCODE plugin.
Results: The present study indicated 428 DEGs, which were utilized for constructing protein-protein interactions with 86 nodes and 166 edges. To understand the reasons behind these 166 interactions, we explored them by sub-network analysis, and three protein complexes revealed scores higher than 5. In order, the function analysis for each cluster indicates its type of association with MS patients. For instance, in cluster 1, the cytokine-cytokine receptor interaction and cell adhesion molecules were reported in more members than 8 down-expressed genes. In addition, cluster 2 showed the Wnt signaling pathway is down-regulated. Furthermore, cluster 3 represents the calcium signaling pathway, which is down-regulated too. So the particular roles of each sub-network are revealed in these macrophage cells, which have a role in MS patients.
Conclusion: In this study, three clusters have been revealed in macrophage cells to indicate their molecular behavior in the MS condition. The first cluster has two important roles: cytokine-cytokine receptor interaction and cell adhesion molecules. The molecular role of cluster two represents the Wnt signaling pathway, and the third cluster indicates the calcium signaling pathway as a molecular role in the macrophage cell in MS patients. These molecular investigations from macrophage cells could improve our knowledge of MS disease and cellular therapeutic approaches in future studies.
Keywords: Multiple Sclerosis, PPIs, Systems Biology, Sub-Networks

Ps-71: The Effect of Human Umbilical Cord Blood Serum on The Expression of Immunomodulatory Genes in Bone Marrow Mesenchymal Stem Cells

Saleh S*, Azarnia M, Moghadasali R, Beirami E, Zeinali H, Mousavizadeh Mobarakeh ZS

Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: salehsotoodeh@gmail.com

Objective: Bone mesenchymal stem cells (BMSCs) are promising for therapeutic applications due to their ease of access, genetic stability, weak immunogenicity, etc. Fetal bovine serum (FBS) has been used as a supplement for cultivation of MSCs,

but it has the contamination risk and adverse immune responses. We studied, human umbilical cord blood serum (HUCBS) in MSC culture and some immunomodulatory properties.
Materials and Methods: BMSCs in the fifth passage were cultured in two groups: control (basic medium + 10% FBS), HUCBS (basic medium + 10% HUCBS). After five days expression rate of interleukin 10 (IL-10), interferon-gamma (IFN-γ), Tumor necrosis factor alpha (TNF-α), and Transforming growth factor beta (TGF-β) were analyzed using real-time PCR.
Results: The expression levels of IL-10, IFN-γ and TNF-α were decreased in the HUCBS group compared to the control group (0.06 ± 0.27 vs. 1.00 ± 0.15; 0.08 ± 0.02 vs. 1.00 ± 0.05; 0.45 ± 0.42 vs. 1.00 ± 0.05 respectively). The level of TNF-α expression was not significantly different between the two groups ($P<0.05$). The expression of TGF-β was increased in the HUCBS group (1.59 ± 0.65) compared to the control group (1.00 ± 0.33).
Conclusion: By reducing the expression of inflammatory genes and increasing the expression of anti-inflammatory genes, HUCBS has affected the immune modulation characteristics of BMSCs and probably can be a suitable alternative to FBS by reducing immune rejection in cell transplantation.
Keywords: Bone Marrow Mesenchymal Stem Cell, Fetal Bovine Serum, Human Umbilical Cord Blood Serum, Immunomodulatory Property

Ps-72: Comparison of Drug Loading in Different Subpopulations of Milk Derived Extracellular Vesicles

Salehi M[1, 2*], Negahdari B[1], Shekari F[2, 3]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, 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. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: faezehshekari@gmail.com

Objective: Extracellular vesicles (EVs) as endogenous transport systems have recently emerged as promising drug delivery vehicles. Since milk is a scalable, available, and safe source of EVs, milk-derived EVs (miEVs) can serve as good alternatives to conditioned medium-derived EVs for this purpose. However, based on our knowledge, no comparative study has been done regarding drug loading in different subpopulations of miEVs, and so was our aim.
Materials and Methods: Here, different subpopulations (35K, 70K, and 100K) of miEVs were isolated by differential ultracentrifugation and characterized by total protein quantification, gel electrophoresis, immunoblotting, and dynamic light scattering (DLS). Then to understand their drug encapsulation efficiency (EE%), an anti-cancer chemotherapeutic drug, sorafenib (SOR) was loaded to miEVs by incubation method. SOR-loaded miEVs were evaluated by spectrophotometer.
Results: Our study indicated that different subpopulations of miEVs could be successfully isolated from the milk. These miEVs were positive for EV-specific markers, CD63, and TSG101, and their average sizes were found to be <200 nm. Using a fixed ratio of miEVs: SOR for each comparison, the EE% of SOR in 35K and 70K miEVs was achieved 20.50 ± 2.50, and 28.67 ± 7.84, respectively. While 100K miEVs exhibited the highest EE% (46.23 ± 3.74) as analyzed by spectrophotometer.
Conclusion: In conclusion, different subpopulations of miEVs are different in terms of drug loading, which can be important in choosing the appropriate subpopulation as a drug carrier.
Keywords: Drug Carrier, Drug Loading, Extracellular Vesicle, Exosome, Milk

Ps-73: Optimization of Cryopreservation Protocol by a Modified Trehalose and Poly (L-Lysine Isophthalamide) to Enhance the Survival and Functionality of Defreezed Rat Pancreatic Islets

Samsonchi Z[1, 2*], Tayebi L[3], Moazenchi M[1, 2], Izadi Zh[4], Hajizadeh-Saffar E[1, 2, 5, 6]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Sciences, Marquette University School of Dentistry, Milwaukee, United States
4. Department of Pharmacology, Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
5. Department of Diabetes, Obesity, and Metabolism, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
6. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: hajizadehehs@gmail.com

Objective: Transplantation of pancreatic islets is curative treatment for type 1 diabetes patients but access to this therapy is limited by temporal mismatch, between availability of pancreas and isolated islets from deceased organ donor, and the recipient's need for freshly isolated islets. To solve this issue, cryopreservation of islets may offer the potential to bank islets for transplant on demand. Current cryopreservation protocols, which use the toxic cryoprotectant dimethyl sulphoxide (DMSO), achieve suboptimal cryosurvival and are not clinically used. Trehalose is a disaccharide and an effective cryoprotectant, decreasing the amount of cell injury by ice crystallization. The aim of this project is to develop an improved and clinically-relevant method for cryopreservation of islets by trehalose and permeabilising biopolymers poly (L-lysine isophthalamide; PLP).
Materials and Methods: PLP polymer was synthetized and characterization tests FTIR and NMR were done. Islets have been isolated and QC tests were done. Isolated islets were cryopreserved and thawed in two groups (DMSO, trehalose + PLP). For determination the effect of freeze media on islet viability and functionality, FDA/PI staining, GSIS assay, DCFDA staining and insulin/glucagon staining were performed. At the end islets were transplanted in diabetic mice and fasting blood glucose (FBS) measurement and intraperitoneal-glucose tolerance test (IP-GTT) were done.
Results: The FTIR and NMR test was confirmed the synthesis of PLP. FDA/PI staining, GSIS test, immunostaining and ROS measurement showed the positive influence of modified cryo-

preservation media on islet. Transplanted islets in diabetic mice could achived normoglycemia in all groups which confirmed there is no negative effect of cryopreservation on islets.
Conclusion: In this study we found that our optimized cryopreservation media which was the combination of trehalose and PLP could increase the viability and functionality of freezed islets.
Keywords: Cryopreservation, Trehalose, Membrane Permeabilizing Biopolymer, Islet, Diabetes

Ps-74: The Effect of Decellularized Extracellular Matrix Derived from Trout Kidney on Mouse Renal Tubular Epithelial Cells

Saniefar H[1, 2*], Abdi Sh[1, 2], Erfanian S[3], Kazemi-Ashtiani M[2, 3], Rajabi S[2, 3], Moghadasali R[1, 2]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Teheran, Iran
2. Department of Developmental Biology, University of Science and Culture, Teheran, Iran
3. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Teheran, Iran
Email: rezamoghadasali@royaninstitute.org

Objective: Nephrogenesis and regeneration potential of kidney in the teleost fish is maintained throughout life. The extracellular matrix (ECM), of trout kidney is probably a major determinant in maintaining the proliferation and differentiation potential of endogenous cells. Here, we investigated the role of trout kidney ECM on mouse renal tubular epithelial cells (TECs).
Materials and Methods: Trout kidney was decellularized and bleached by SDS, Triton X-100 and bleaching buffer containing H_2O_2. Decellularized tissue was characterized by DNA quantification, BCA, collagen and sGAG assay, H&E, DAPI, Alcian blue and Sirius red staining. Culture dishes were coated by rat tail collagen and decellularized ECM. Cytotoxicity was indirectly perused by MTS. The TECs were isolated from fvb/n mouse kidney by enzymatic digestion. Cell viability and proliferation were investigated by MTS and Live/Dead assay. Tjp1, Cdh1 as epithelial markers, and Vim and Mmp9 as mesenchymal markers gene expression was evaluated using qRT-PCR.
Results: DNA quantification revealed that DNA was removed significantly (P value< 0.0001). The loss of collagen and sGAG after decellularization was not significant (P value>0.05). The coating materials were not cytotoxic. The proliferation of TECs during 7 days was significantly increased compared to the control group (P value< 0.0358). Moreover, gene expression evaluation showed epithelial-mesenchymal transition (EMT) in these cells.
Conclusion: In this study, fish kidney ECM has been decellularized and characterized successfully for the first time. This ECM can be an appropriate source for future studies based on kidney organogenesis and tissue engineering applications.
Keywords: Extracellular Matrix, Epithelial-Mesenchymal Transition, Proliferation, Renal Tubular Epithelial Cells, Trout

Ps-75: The Effect of Placenta-Derived Mesenchymal Stem Cells on Folliculogenesis and Ovarian Histomorphology in Letrozole-Induced Polycystic Ovary

Sarvestani M[1, 2*], Rajabzadeh A[3, 4], Ghavipanjeh Gh[1, 2], Mazochi T[4]

1. Department of Physiology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran
2. Institute for Basic Sciences, Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran
3. Department of Applied Cell Sciences, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
4. Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran
Email: m.sarvestani76@gmail.com

Objective: Polycystic ovary syndrome (PCOS) is a reproductive and metabolic disease that has affected many women in the world, and there is still no definitive cure for it. the use of mesenchymal stem cells (MSCs) for treating PCOS has expanded due to their diverse and multifaceted therapeutic effects, including the ability to repair tissues and their paracrine effects. Placenta-derived mesenchymal stem cells (PD-MSCs) are derived with wide sources, fewer ethical controversies, and low immunogenicity, and are considered the best choice for stem cell therapy. this study was designed for evaluating effect of (PD-MSCs) on Folliculogenesis in rat model of PCOS.
Materials and Methods: In this experimental research, 15 female wistar rat (200-250 g) were divided in to three groups (n=5) including, control, PCOS, PCOS+PD-MSCs (1×10^6). On days 14 post cell transplantation, the ovaries are removed from the body and histological serial sections into 5 µm thickness were prepared by microtome and stained with hematoxylin-eosin and the number of follicles in each group was compared.
Results: The result showed The number of cystic follicles in the PCOS+PD-MSCs group (1.8 ± 1.7, $P<0.0001$) significantly decreased compared to the PCOS group (13.4 ± 1.1, $P<0.0001$) and the number of corpus luteum in the PCOS+PD-MSCs (12.4 ± 2.0, $P<0.0001$) significantly increased compared to the PCOS group (5.4 ± 1.1, $P<0.0001$)
Conclusion: our results suggest that transplantation of placenta- derived mesenchymal stem cells (PD-MSCs) able to regulate folliculogenesis in PCOS model.
Keywords: Folliculogenesis, Mesenchymal Stem Cells, Polycystic Ovary Syndrome

Ps-76: Evaluation of Different Strategies in Extracellular vesicles miRNA Loading

Seydi H[1*], Bahadori S[1], Shekari F[2], Vosough M[1]

1. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, 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: massoudvosough@gmail.com

Objective: One of the most common malignancies in human is hepatocellular carcinoma (HCC). Non-coding RNA molecules called miRNA are crucial for regulation of biological activities. It appears that certain miRNA has significant regulatory functions in liver cancer. A mass of research indicated that miRNA-29a-3p has a lower expression in liver cancer cells in comparison with non-cancerous cells. In addition, miRNA-29a-3p overexpression increases the sensitivity to Gemcitabine treatment in pancreatic cancer cell line. Extracellular vehicles (EVs) are promising delivery vehicles for various genetic therapeutics.

Materials and Methods: In the current study, we aimed to load miRNA-29a-3p inside the Human Wharton Jelly Mesenchymal Stem Cells derived EVs (hWJ-MSC-EVs) with Calcium chloride ($CaCl_2$), heat shock and electroporation methods. In this study, hWJ-MSC-EVs were isolated by differential centrifugation (20,000 g and 100,000g) methods and then was characterized for EV specific protein markers using western blotting, SEM imaging and size distribution (DLS). In the second step, EVs was loaded with miRNA-29a-3p by electroporation (400V, 150 µF, 2 pulses, 30 ms,10 ms pause) and $CaCl_2$- heat shock.
Results: The encapsulation efficiency by the $CaCl_2$ (0.1 M)-heat shock method was 8.26%, while in electroporation it was 3.5%.
Conclusion: This idea indicated that application of $CaCl_2$ strategy might be a useful method for enhancing the loading of miRNA molecules into EVs.
Keywords: $CaCl_2$ Electroporation, Extracellular Vesicles, Hepatocellular Carcinoma, MiR-29a-3p, Strategy

Ps-77: A Cost- and Time-Effective Protocol for Scalable Generation of Human Embryonic Stem Cell-Derived Cardiac Fibroblasts

Shafaghi M[1*], Pahlavan S[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 Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Email: sarapahlavan@royaninstitute.org

Objective: The mammalian heart comprises various cell types, which play an important role in cardiac physiology. According to previous studies, the cardiac muscle cells have the highest cell density compared to other types and include about 50% of heart tissue. Other cell types include smooth muscle cells (SMC), endothelial cells (EC), cardiac fibroblasts (CF) and immune cells, among which cardiac fibroblasts have the highest cell density after cardiac muscle cells. CF comprises about 30% of the heart tissue and play a key role in the form and function of this organ. In addition to physiology, CF contributes to the cardiac pathology by secretion of the excessive extracellular matrix after being activated during cardiac injuries. Therefore, CF might be an important therapeutic target in cardiovascular diseases. In this study, we aimed to introduce a cost- and time-effective, universal and scalable protocol for *in vitro* generation of cardiac fibroblasts to use for developmental, physiological and pathological, as well as tissue engineering studies.
Materials and Methods: Human embryonic stem cells (hESC) were subjected to step-wise differentiation into cardiac progenitor cells, epicardial cells and cardiac fibroblasts by small molecule (CHIR99021, SB431542, Purmorphamine) and growth factor (bFGF) treatment.
Results: The resulting cells were characterized for CF marker, vimentin, which resulted in more than 90% vimentin+ CF. The doxorubicin stimulation of hESC-derived CF resulted in myofibroblast trans-differentiation with majority of CFs turning to large, SMA+ cells. Furthermore, collagen assay showed higher expression and secretion of this protein after CF activation by doxorubicin. These results could be observed by CFs at higher passages and after bio banking as well.
Conclusion: Therefore, this CF differentiation protocol can be used for future *in vitro* studies of cardiac tissue, disease modeling and drug discovery.
Keywords: Cardiac Fibroblast, Differentiation, Human Embryonic Stem Cell, Protocol

Ps-78: PC12 Cell Line Co-Culture with Adipose-Derived Stem Cells Results in Effective Myogenic Differentiation

Shafiei Seifabadi Z*, Dayer D , Bayati V

Department of Anatomy, Cellular and Molecular Research Center, Ahvaz, Iran
Email: Z.shafiei67@gmail.com

Objective: Several studies have suggested the administration of mesenchymal stem cells for functional skeletal muscle tissue regeneration. In the current study, The effect of PC12 cell line co-culture with ADSCs on the myogenic differentiation of Adipose-derived stem cells (ADSCs) was investigated.
Materials and Methods: The ADSCs were cultured within a myogenic differentiation medium (5-aza (3 g/ml) and 5% horse serum in DMEM-HG) for 7 days. The differentiated ADSCs were then co-cultured with various percentages of PC12 (30, 50, and 70%) for another 7 days. A real-time PCR technique was utilized to assess gene expression. Western blotting was used to determine protein expression.
Results: The differentiated ADSCs had significantly higher levels of MYH1, MYH2, and Chrn-γ gene expression in comparison to undifferentiated ADSCs ($P<0.0001$). When differentiated ADSCs were co-cultured with varying percentages of PC12, the gene expression of MYH1, MYH2, and Chrn-γ was significantly higher than in differentiated ADSCs ($P<0.001$). ADSCs co-cultured with 50% PC12 had significantly higher MYH protein expression than the control group ($P<0.01$). The nACHR protein expression was significantly increased in ADSCs that were co-cultured with 50% of PC12 compared to the differentiated ADSCs group ($P<0.001$).
Conclusion: Finally, it can be concluded that the co-culture of PC12 with ADSCs stimulates myogenic differentiation. The percentages of PC12 had a substantial effect on the degree of myogenic differentiation.
Keywords: Adipose Derived Stem Cells, ADSCs, Co-Culture, Myogenic Differentiation, PC12 Cell Line

Ps-79: The Expression of Embryonic and Adult Subunits of Nicotinic Acetylcholine Receptors is Influenced by The Myogenic Differentiation of Rat Adipose-Derived Mesenchymal Stem Cells

Shafiei Seifabadi Z[1*], Dayer D[2], Bayati V[1], Azandeh SS[1], Rashno M[2]

1. Department of Anatomy, Cellular and Molecular Research Center, Ahvaz, Iran
2. Medical Basic Sciences Research Institute, Cellular and Molecular Research Center, Ahvaz, Iran
Email: Z.shafiei67@gmail.com

Objective: The nicotinic acetylcholine receptor (nAChR) is a crucial protein in the neuromuscular junction (NMJ). ADSC differentiation into muscle cells provides a useful model for investigating the molecular mechanisms of NMJ development and synaptic maturation. The purpose of this work was to investigate the expression of embryonic and mature nAChR subunits throughout the myogenesis of rat ADSCs.

Materials and Methods: The stem cells were isolated from rat adipose tissue using the enzymatic digestion procedure. The cells were cultured in DMEM-HG contained 10% FBS in 5% CO2 and 37º c. Myogenic differentiation was carried out using 5-aza (3g/ml) and 5% horse serum through a 28 days differentiation protocol. The expression of the nAChR - ε and nAChR- γ subunits was measured on days 3, 7, 14, and 21 of differentiation. Protein expression was investigated by western blotting.
Results: Following myogenic differentiation of ADSCs, the expression of both emberyonic (nAChR- γ) and mature (nAChR- ε) subunits increased significantly ($p < 0.0001$). The expression of the nAChR- ε subunit showed a significant increase compared to the nAChR- γ subunit ($p < 0.05$). The highest amount of the nAChR- ε subunit was expressed on the 21st day of differentiation ($p < 0.0001$).
Conclusion: According to the research, myogenic differentiation of ADSCs using horse serum and 5-aza can produce functional myogenic cells with NMJ potential.
Keywords: Nicotinic Acetylcholine Receptor, Neuromuscular Junction, NMJ, Rat Adipose-Derived Stem Cells, Myogenic Differentiation

Ps-80: PC12 Cell Line Co-Culture with Adipose-Derived Stem Cells Results in Effective Myogenic Differentiation

Shafiei Seifabadi Z[1, 2*], Dayer D[2], Bayati V[1], Azandeh SS[1], Rashno M[2]

1. Department of Anatomy, Cellular and Molecular Research Center, Ahvaz, Iran
2. Medical Basic Sciences Research Institute, Cellular and Molecular Research Center, Ahvaz, Iran
Email: Z.shafiei67@gmail.com

Objective: Several studies have suggested the administration of mesenchymal stem cells for functional skeletal muscle tissue regeneration. In the current study, the effect of PC12 cell line co-culture with ADSCs on the myogenic differentiation of Adipose-derived stem cells (ADSCs) was investigated.
Materials and Methods: The ADSCs were cultured within a myogenic differentiation medium (5-aza (3 g/ml) and 5% horse serum in DMEM-HG) for 7 days. The differentiated ADSCs were then co-cultured with various percentages of PC12 (30%, 50%, and 70%) for another 7 days. A real-time PCR technique was utilized to assess gene expression. Western blotting was used to determine protein expression.
Results: The differentiated ADSCs had significantly higher levels of MYH1, MYH2, and Chrn-γ gene expression in comparison to undifferentiated ADSCs ($P<0.0001$). When differentiated ADSCs were co-cultured with varying percentages of PC12, the gene expression of MYH1, MYH2, and Chrn-γ was significantly higher than in differentiated ADSCs ($P<0.001$). ADSCs co-cultured with 50% PC12 had significantly higher MYH protein expression than the control group ($p<0.01$). The nACHR protein expression was significantly increased in ADSCs that were co-cultured with 50% of PC12 compared to the differentiated ADSCs group ($P<0.001$).
Conclusion: Finally, it can be concluded that the co-culture of PC12 with ADSCs stimulates myogenic differentiation. The percentages of PC12 had a substantial effect on the degree of myogenic differentiation.
Keywords: Adipose Derived Stem Cells, ADSCs PC12 Cell Line, Co-Culture, Myogenic Differentiation

Ps-81: Comparison of Umbilical Cord Serum and Human Platelet Lysate and The Synergistic Effect of Nano Curcumin and Crocin as Supplements with FBS in Mesenchymal Stem Cell Culture

Sheikh A[1, 2*], Baharara J[1, 2], Kaffash Farkhad N[1, 2], Jafari MR[1,2], Kerachian MM[1, 2], Tavakol Afshari J[1, 2]

1. Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran, Immunology Research Center of Mashhad Medicine University, Mashhad, Iran
2. Immunology Research Center, Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: akram1357.sh@gmail.com

Objective: Fetal Bovine Serum (FBS) is the most common element in mesenchymal stem cell (MSC) culture. It is rich in growth factors and contains a few amounts of gamma globulin; however, FBS can transfer 7-30 mg of bovine protein per 107 cells to the host and cause an immunological reaction. Therefore, finding suitable alternatives can help the enrichment of the media. This study was designed to compare umbilical cord serum and human platelet lysate and evaluate the synergistic effect of nano curcumin and crocin as supplements with FBS to enrich MSCs culture.
Materials and Methods: MSCs were isolated from Wharton's jelly and cultured in α-MEM medium supplemented with FBS and antibiotics. After expansion of MSCs, they were studied in 12 groups. MTT test was performed to check the cell proliferation and cell survival after 48h and 72h. Population doubling time (PDT) was reported after 48h and 72h. The effect of curcumin and crocin on cell apoptosis was investigated with the Annexin V kit through the flow cytometry technique. The expression levels of Nanog, Sox2, and Oct4 genes were evaluated through RT-qPCR.
Results: The proliferation of MSCs was significantly higher in culture media containing UCS and HPL than in the FBS (control) group after 72h. On the other hand, the proliferation rate significantly decreased in culture media containing UCS and UCS+FBS (according to PDT and cell counting) compared to the control group. Cell apoptosis was significantly higher in all culture media containing 0.3µm nano curcumin than in the control group. However, 2.5µm crocin caused a significant decrease in cell apoptosis compared to the control group. The expression of pluripotency genes in HPL and crocin groups increased significantly compared to the control group.
Conclusion: The results imply the beneficial consequence of UCS and HPL in the proliferation of MSCs for therapeutic strategies. Also, crocin effects in combination with UCS and HPL in cell survival and reducing cell apoptosis should be considered.
Keywords: Crocin, Human Serum, Mesenchymal Stem Cell, Nano Curcumin,

Ps-84: Indirect Hypoxic Clonal Mesenchymal Stromal Cell-Derived Extracellular Vesicles Attenuate the Immune Responses in The Type 1 Diabetes Model

Soleymani Goloujeh M[1, 2, 3*], Shekari F[2, 3, 4, 5], Hassani SN[2, 3], Hajizadeh-Saffar E[3, 6]

1. Department of Applied Cell Sciences, Faculty of Basic Sciences and Advanced Medical Technologies, Royan Institute, 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. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Molecular Systems Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
6. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: en.hajizadeh@royan-rc.ac.ir

Objective: Cellular therapy using mesenchymal stem cells (MSCs) has been identified as a recent therapeutic approach for the treatment of Type 1 diabetes (T1D). Studies have revealed that adopting an alternative approach for modulating the immune system, such as MSC-derived extracellular vesicles (EVs), may be more effective than cell therapy due to the challenges involved in cell therapy. MSC preconditioning with various procedures appears to be a promising method to improve their therapeutic effects. For this reason, we evaluated the effects of hypoxia-preconditioned MSC-derived EVs on immune responses in a multiple low-dose streptozotocin (MLD/STZ)-induced T1D mouse model and compared them to their source cells.
Materials and Methods: Four pretreatments were administered indirectly to cMSCs along with their respective controls. Indirect treatments included four groups: IFN-γ (50 ng/ml), Poly(I:C) (42.22 μg/ml), LPS (1 μg/ml), and Hypoxia (1% oxygen) treatments applied to peripheral blood mononuclear cells (PBMCs) and conditioned medium (CM) were used to MSCs in order to mimic *in vivo* conditions. A suitable pretreatment was selected based on the selection criteria (cytokine assay, angiogenesis, and inhibition of lymphocyte proliferation). The EVs were isolated from the CM of the chosen group via ultracentrifugation and characterized. Animal studies were conducted on MLD/STZ-induced T1D mice. Four treatment groups were prepared: indirect hypoxia-induced MSC-derived EVs (HY-PB-MSC-EVs) and cells derived from them (HY-PB-MSCs). In the end, the animals were monitored 73 days after the second injection. The effects of each treatment on immunomodulation and regenerative capacity in the T1D model were assessed.
Results: An *in vitro* study has demonstrated that indirect hypoxia treatment is the most effective among all pretreatments. According to an *in vivo* study, fasting blood glucose (FBG) levels decreased in treatment groups compared to a sham group. Nevertheless, indirect hypoxia-induced MSC-EVs increased serum levels of anti-inflammatory cytokines (IL-4 and IL-10) in MLD/STZ-induced mice models in comparison with their source cells. Moreover, indirect hypoxia-induced MSC-EVs attenuate immune responses in MLD/STZ-induced mice models. Furthermore, MSC-EVs induced by indirect hypoxia decreased pro-inflammatory cytokines (TNF-α and IL-6) in the sera of MLD/STZ-induced mice models in comparison with their source cells.
Conclusion: Our study showed that the immunomodulatory potential of indirect hypoxia-induced MSC-EVs is considerably higher than their source cells. These findings confirmed the effectiveness of indirect hypoxia treatment as a valuable pretreatment regimen of MSCs to modulate immune responses in T1D models. Consequently, our findings would suggest that indirect hypoxia-induced MSC-EVs with little modification could be a potential option for attenuating of immune responses in T1D.
Keywords: Extracellular Vesicles, Immunomodulation, Mesenchymal Stromal Cells, Preconditioning, Type 1 Diabetes

Ps-83: Development of Acellular Human Skin for Fetal Treatment of Myelomeningocele: An *In Vivo* Study

Soltani Khaboushan A*, Majidi Zolbin M, Kajbafzadeh AM

Pediatric Urology and Regenerative Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: masoumeh.majidizolbin@gmail.com

Objective: Myelomeningocele is a severe form of spina bifida that affects 1 in every 1000 live births worldwide. This condition can result in a range of complications, including paralysis, bladder and bowel dysfunction, and mental health issues (1, 2). Recent research has indicated that fetal treatment of myelomeningocele can provide better outcomes than postnatal (3, 4). However, this approach presents certain challenges, such as a shortage of tissue for covering lesions, incomplete regeneration, and complex surgical procedures (2). We propose a tissue engineering-based solution that can enhance the effectiveness of fetal treatment of myelomeningocele, which may improve patient outcomes and quality of life.
Materials and Methods: The harvested skin tissue was frozen and thawed before the application of the decellularization process. Sodium dodecyl sulfate (SDS) and Triton X-100 were used for decellularization. H&E and DAPI staining were used to confirm the effectiveness of the protocol. The lack of toxicity of the tissue and proliferation of cells on the tissue was confirmed via the MTT test. The scaffold was transplanted via fetal surgery on the defect in the rat model of myelomeningocele. IHC evaluation for CK 5/6 was done 4 days later at term to detect keratinocytes.
Results: Histopathologic evaluations confirmed the effectiveness of the decellularization process. The MTT test showed that the absorbance was not different in the tissue group compared to controls ($P>0.05$ in all 3 time points), and tissues do not cause cell toxicity. Moreover, the proliferation of cells was desirable on the scaffold, and it was significantly higher on day 7 compared to day 5 ($P<0.05$). Appropriate coverage of defect was observed by gross evaluation of defect. IHC evaluations demonstrated the epithelization of the intrauterine transplanted scaffold.
Conclusion: Here we successfully introduced a protocol for effective decellularization of human skin, creating a scaffold that could serve as a potential construct for the fetal treatment of myelomeningocele lesions.
Keywords: Decellularization, Fetal Surgery, Myelomeningocele, Regenerative Medicine, Tissue Engineering

Ps-84: Improving Cell Growth and Proliferation Using Conductive Polyaniline-Based Nanofibrous Wound Dressing

Tajedin R*, Daraeinejad Z, Shabani I

Department of Biomedical Engineering, Amirkabir University, Tehran, Iran
Email: shabani@aut.ac.ir

Objective: Conductive wound dressing accelerating the pro-

cess of regeneration, healing, preventing possible infections in the wound bed and play an effective role in regulating cell behaviors. The goal of this research was to investigate the behavior of fibroblast cells cultured on conductive polylactide (PLLA; as the carrier)/ polyaniline (PANI)-brilliant blue (BB; as an antibacterial dopant) nanofibrous wound dressing.
Materials and Methods: Two different dopants were used for fabrication of PLLA/PANI nanofibers, including BB and camphor sulfonic acid (CSA). Uniform and beadles nanofibers were prepared after optimization of electrospinning parameters (high voltage power supply:30 kV, an air gap distance:30 cm, flow rate: 0.002 ml/h). The conductive wound dressings were characterized using Energy Dispersive X-ray spectroscopy/ mapping (EDX), scanning electron microscopy (SEM) and cell culture study.
Results: The EDX test was used to confirm the uniform presence of doped PANI in the entire nanofibrous structure. The morphology of PLLA/PANI-BB nanofibers was homogenous and beadles and the conductivity of this wound dressing was around 272.8 S/m. The results of MTT assay and cellular SEM showed the excellent Adhesion, growth, proliferation and viability of Human Dermal Fibroblasts (HDF) cells on PLLA/ PANI-BB wound dressing.
Conclusion: The prepared PLLA/PANI-BB wound dressing is suitable for many types of wounds due to its nanofibrous morphology and electrical conductivity. Furthermore, antibacterial property of the wound dressing makes it a good option for wound healing.
Keywords: Polyaniline, Wound Dressing, Dopant, Conductive Nanofibers, Wound Healing

Ps-85: The Effect of Mesenchymal Stem Cells-Derived Extracellular Vesicles Containing TAK242 on Inflammatory Status of Mesenchymal Stem Cells Primed with Interferon Gamma *In Vitro*

Talebi Jouybari M[1, 2*], Taghiyar L[1], Baghaban Eslaminejad MR[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
Email: leilataghiyar@royaninstitute.org

Objective: It has been demonstrated that inhibiting the activation of toll-like receptors (TLRs) can hinder inflammation disease progression. TAK242, a small molecule inhibitor of TLR4, has been employed in various autoimmune diseases such as rheumatoid arthritis (RA) and sepsis. Additionally, utilizing extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) presents another promising therapy due to their immunomodulatory effect. So, this study aimed to optimize the TAK242 half-life and evaluate the synergistic effect of EVs-loaded TAK242 for reducing inflammation.
Materials and Methods: Human bone marrow-derived MSCs (hBM-MSCs) and EVs were obtained from the ATMP center at Royan Institute in Iran and characterized to confirm their properties. The cellular uptake of the EVs was confirmed using PKH26-labeled MSCs and Calcein AM-labeled EVs. TAK242 was loaded into the EVs using electroporation, and the encapsulation efficiency was estimated. The toxicity of TAK242 was evaluated using an MTT assay in hBM-MSCs primed with IFN-γ. The inflammatory gene and cytokine expression in hBM-MSCs were evaluated by Real-time PCR and ELISA techniques after incubating them with TAK242 and TK242-loaded EVs for 48 hours.
Results: Outcomes showed that TAK242 was efficiently loaded into the EVs, with an encapsulation efficiency of 11.15%. The optimal dose of TAK242 was found to be 60 nM. Real-time PCR and ELISA results demonstrated that TAK242 and EV-TAK242 treatments significantly reduced the expression of inflammatory cytokines IL-1β, IL-6, and TNF-α in hBM-MSCs compared to the control group. Transcriptomics analysis also demonstrated a significant decrease in TLR4-specific inflammatory pathway genes (TLR4 and MYD88) expression in the TAK242 and EV-TAK242 groups relative to the control group.
Conclusion: The findings suggest that TAK242 delivered by EVs could serve as a targeted and more effective therapy for treating RA.
Keywords: Anti-Inflammatory Genes, Extracellular Vesicles, Mesenchymal Stem Cells, Rheumatoid Arthritis, TAK242

Ps-86: Msh Homeobox1-Transduced Bone Marrow Mesenchymal Stem Cells-Derived Extracellular Vesicles Induce Proliferation, Migration, and Osteogenic Differentiation *In Vitro* and Digit Tip Regeneration in an Amputee Mice Model

Talebi Jouybari M[1, 2*], Shemshadi S[1, 2], Baghaban Eslaminejad M[1], Taghiyar L[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
Email: leilataghiyar@royaninstitute.org

Objective: Limb regeneration is limited in adult mammals due to a lack of blastemal cell (BC) formation and subsequent regenerative signaling pathway induction. Extracellular vesicles (EVs) are powerful biological tools that can be used as an alternative to manipulated cells for human clinical approaches, without ethical concerns. In our previous study, we created blastema-like cells (BLCs) by overexpressing MSXs (Hox7 & Hox8) genes in mouse bone marrow-derived mesenchymal stem cells (mBMSCs). Therefore, the aim of this study is to use BLCs-derived extracellular vesicles (BLCs-EVs) to assess mBMSCs proliferation, migration, and osteogenic differentiation *in vitro*, as well as analyze the therapeutic effects of BLCs-EVs on digit tip regeneration in mice.
Materials and Methods: BLCs and non-induced mBMSCs were cultured and expanded, and the cell-conditioned medium was harvested for EV isolation by ultracentrifugation. The EVs were then confirmed for size, morphology, and CD81 expression by flow cytometry, DLS, and SEM assessments. MSX1 and related proteins such as MSX2, FGF8, and BMP4 were analyzed using the western blot technique. The uptake of BLCs-EVs by mBMSCs was confirmed by immunostaining assessment. Cell proliferation, migration, and osteogenic activity were examined in mBMSCs treated with BLCs-EVs using MTT, scratch, and real-time PCR analysis. Finally, the regenerative potential of BLCs-EVs, hBM-MSCs-EVs, and a sham group (injected with normal saline) was examined in mice with

digit tip amputations.
Results: We found that BLCs-EVs increase migration, proliferation, and osteogenesis of mBMSCs *in vitro*. More importantly, BLCs-EVs increased mice digit tip regeneration by new bone induction and nail formation. In mBMSCs-EV formed abnormal bones and nails, and in the sham group, the presence of new tissue wasn't observed.
Conclusion: The findings suggest that BLCs-EVs could serve as a powerful tool for digit regeneration in human amputees.
Keywords: Bone Differentiation, Digit Tip Regeneration, Extracellular Vesicles, MSX1 Gene, Proliferation

Ps-87: The Potential of Embryonic Cerebrospinal Fluid on The Differentiation of Hair Follicle Stem Cells into Dopaminergic Neurons

Tavakoli Z[1, 2*], Jameii F[1, 2], Dianatpour M[1, 2], Salehi MS[3], Pandamooz S[2]

1. Department of Medical Genetics, Shiraz University of Medical Sciences, Shiraz, Iran
2. Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: sareh_pandamooz@sums.ac.ir

Objective: Cerebrospinal fluid (CSF) is a vital body fluid, produced by choroid plexus (CP) and flows into brain ventricles. Due to the presence of signaling molecules and growth factors, CSF has a crucial role in supporting the development of the central nervous system (CNS) regulating neurogenesis, differentiation, and proliferation. Hair follicle stem cells (HFSCs) found in the bulge of the hair follicle, are capable of transforming into neural cells and have been extensively studied in CNS conditions. This is the first study in which the influence of CSF on the conversion of HFCs into dopaminergic neurons was investigated.
Materials and Methods: Embryonic CSF was collected from the cisterna magna of gestational day 19 rat embryos and its quality was assessed with cell counting and BCA assay. The HFSCs were isolated from the bulge region of hair follicles and were cultured in α-MEM supplemented with 10% fetal bovine serum and 10% chicken embryo extract. e-CSF was added to the media at 20% concentration. MTT assay was carried out to define cell viability and neurite length was measured. The expression of pertinent markers was assessed at the gene level with RT-PCR.
Results: The rate of proliferation in isolated EPI-NCSCs was increased. Moreover, morphological change was seen following treatment with e-CSF. The expression of β–tubulin III (early neuronal marker) and tyrosine hydroxylase (TH) (dopaminergic neuron marker) elevated in the presence of e-CSF. Also, doublecortin (DCX) (stemness marker) expression was decreased.
Conclusion: Our findings demonstrate that e-CSF can promote the fate of HFCs towards neural lineage. These findings suggest that e-CSF, owing to its content, can be beneficial in generating dopaminergic neurons.
Keywords: Cerebrospinal Fluid, Dopaminergic Neurons, Hair Follicle Stem Cells

Ps-88: Study Locomotor Improvement in Spinal Cord Injury Rat Model upon Cis-p Tau Elimination

Vahedi A

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

Objective: Spinal cord injuries are devasting. It has been estimated that between 2-3 million people live with disabilities related to SCI. Also, hyperphosphorylation of Tau protein plays a main role in Neurodegeneration. In addition, cis p-Tau has been expressed significantly in SCI patient and could spread from side of injury to other areas of spinal cord as well as to the brain.
Materials and Methods: Male Wistar rats (250-280g) were obtained and housed in clear plastic cages, under controlled temperature and humidity in 12 hours light/dark cycle with free food and water access. For moderate to severe SCI modeling, we used combination of dorsolateral laminectomy and spinal cord contusion at T8-T9 segment. NYU impactor was used for contusion (10g weight was dropped to spinal cord from height 25 mm) Treatment: Monoclonal Cis-p Tau antibody was used to diminish cis p-tau. 1 mg/kg antibody was injected IV for 28 days. in one week, every two-day, second week every 4 days and in week 3 and 4, single dose in week. Locomotor Analysis: To evaluate the efficacy of cis mAb in treating SCI, motor function was assessed by BBB test. In BBB, animals were placed individually in open-field chamber and allowed to freely explore for 5 minutes and each animal got score from 0 to 21.
Results: BBB Score in treatment group was 7 to 8, while in treatment group, animals got higher score (14 to 15).
Conclusion: BBB data analysis showed a significant locomotor improvement in treated animals in compare with placebo group.
Keywords: Cis mAb, Spinal Cord Injury, Tau Protein,

Ps-91: Developing A Thermosensitive Scaffold for Controlled Delivery of Retinoic Acid in Testis Tissue Engineering

Zarei H[1, 2, 3*], Movahedin M[1, 2, 3], Ganji F[1, 2], Ghiaseddin A[3]

1. Department of Anatomical Sciences, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Biotechnology Group, Faculty of Chemical Engineering, Tarbiat Modares University, Tehran, Iran
3. Adjunct Research Associate Professor at Chemistry Department, Michigan State University, East Lansing, MI, United States
Email: Hooman1634zarei@gmail.com

Objective: Releasing scaffolds holds promise for testes tissue engineering. Chitosan (CS) is a natural biopolymer widely used as a scaffold and delivery system, while decellularized testis matrix provides a natural extracellular matrix-like structure. This study aimed to develop a new thermosensitive scaffold composed of CS hydrogel and testis decellular plates (TDPs) for controlled All-trans retinoic acid (ATRA) delivery.
Materials and Methods: Mouse testes underwent SDS 1% treatment and were evaluated for decellularization efficacy using morphological, DNA content, and DAPI staining assessments. Thin plates of decellularized testes were combined with a thermosensitive scaffold created using CS hydrogel, beta-glycerophosphate (bGP), and varying ATRA concentrations. The designed scaffold underwent evaluation for gelation time, morphology, and wettability. An *in vitro* ATRA release study

was conducted at 34°C, and the cytocompatibility of the scaffold was also assessed.
Results: Decellularization effectively removed cells while preserving the extracellular matrix (ECM). Chitosan/bGP hydrogels were prepared using different formulations, and increasing the bGP concentration significantly reduced gelation time ($P<0.05$). Adding ATRA did not significantly affect hydrogel hydrophilicity. *In vitro* release studies showed sustained drug release behavior with an initial low burst, and increasing ATRA concentration decreased the drug release rate. The scaffold was found to be non-toxic and biocompatible.
Conclusion: The new thermosensitive scaffold has potential for use in *in vitro* spermatogenesis and testis tissue engineering by sustainably delivering ATRA. The scaffold can provide a favorable environment for cell proliferation and differentiation and may be useful in developing new therapies for male infertility and other tissue injuries or diseases.
Keywords: Chitosan, Hydrogel, Releasing Scaffold, Retinoic Acid

Ps-90: Fabrication and Characterization of Electrospun Polycaprolactone Nanofiber Loaded with AgNPs Placed on Human Amniotic Membrane for Volumetric Muscle Loss

Zohour Soleimani M[1, 2*], Bayati V[2], Nejaddehbashi F[2], Orazizadeh M[2], Khoshnam SE[2]

1. Department of Anatomical Sciences, School of of Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Anatomical Sciences, Medical Basic Sciences Research Institute, Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
Email: m.zohorsoleimani@gmail.cm

Objective: Volumetric muscle loss (VML) is the traumatic, degenerative, or surgical loss of muscle tissue, which may cause change in loss of function and physical deformity. Silver nanoparticles (AgNPs) are known antimicrobial agents against a wide range of microorganisms. This study investigates the design and fabrication of electrospun polycaprolactone (PCL) nanofiber loaded with AgNPs placed on human amniotic membrane (HAM) for VML.
Materials and Methods: In this experimental study, electrospun nanofibrous composed of PCL, PCL/AgNPs, PCL placed on HAM, PCL/AgNPs placed on HAM were prepared. After the fabrication of scaffolding, scaffolds were characterized *in vitro* by physical properties, antimicrobial properties.
Results: According to *in vitro* results, cells adhered and proliferated in all scaffolds. PCL/HAM/AgNps and PCL/HAM scaffolds showed remarkable enhancement in tensile strength compared to HAM scaffod.
Conclusion: PCL/HAM/AgNps, PCL/AgNPs , PCL/HAM scaffolds could be considered as promising types of skeletal muscle scaffold in VML *in vivo*.
Keywords: Volumetric Muscle Loss, Polycaprolactone, Human Amniotic Membrane, Silver Nanoparticles, Electrospinning

Authors Index

BEHRAD
ROOYESH ROYAN

شرکت دانش بنیان بهراد رویش رویان

اولین تولیدکننده کتتر انتقال جنین در ایران

نیدل پانکچر
Ovum Aspiration Needle

کتتر انتقال جنین
Embryo Transfer Catheter

کتتر IuI
Iul Catheter

کتتر نمونه برداری آندومتر
Endometrial Suction Catheter

Behrad_Medical
info@BehradMedical.ir
۰۲۱ - ۷۷ ۶۰ ۵۱ ۶۷
www.behradMedical.ir

خط مستقیم واحد فروش ۰۲۱ - ۹۱ ۳۰ ۹۷ ۴۷

Taking a closer look, there may be more patients who could benefit from LH

Severe FSH and LH deficiency is multifactorial.[1] It can be congenital or acquired, permanent or transient – potentially contributing to a suboptimal response.[1-3]

Look twice to identify patients who may benefit from Pergoveris®

Pergoveris®, the first and only recombinant hFSH:hLH[4,5]

References: 1. Hayes F, et al. Hypogonadotropic Hypogonadism (HH) and Gonadotropin Therapy. 2000. In: Endotext. South Dartmouth (MA): MDText.com, Inc. 2. Rinaldi and Selman. Int J Womens Health 2016. 8:169–179. 3. Kol and Homburg. Human Reproduction 2008. 23;5:1004–1006. 4. Leao R de BF, Esteves SC. Clin Sao Paulo Braz 2014;69:279–293. 5. Pergoveris®. Summary of Product Characteristics. July 2020.

Refer to the locally approved PIL in the country of dissemination before prescribing.
For full information please contact: Merck Serono Middle East FZ-Ltd., P.O. Box 22730, Dubai, UAE.
Phone +971 4 375 2700, Fax +971 4 429 1390, www.merckgroup.com

NE-PER-00010

MERCK

Fefol® Family USA

Original Iron supplement Brand in the world

The Licensed Iron Supplement Trusted Across the UK & EU& USA

OTDP Quality Life Pharmaceuticals