Oral Presentations

11th Yazd International Congress and Student Award on Reproductive Medicine

O-1

The use of machine learning for human sperm and oocyte selection and success rate in in vitro fertilization methods

Amini Mahabadi J[1], Enderami E[2], Nikzad M[1, 3], Hassani Bafrani H[3].

1.*Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
2.*Immunogenetics Research Center, Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
3.*Anatomical Sciences Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran.*

Email: amini-ja@kaums.ac.ir

Infertility is indeed a significant global health concern. The quality of gametes plays a pivotal role in determining the success rates of assisted reproductive technology (ART) cycles. In contemporary fertility and reproductive medicine, the utilization of machine learning (ML) has emerged as a powerful tool for processing large datasets, offering the potential to enhance existing ART practices. Therefore, the aim of this study was to evaluate the use of ML for human sperm and oocyte selection and success rate in in vitro fertilization methods. The objective of this narrative review study was to assess sperm and oocyte characteristics in humans using ML techniques. This approach can contribute to a more precise evaluation of the gamete, leading to improved decision-making and potentially higher success rates in ART procedures. Using ML abilities, researchers can obtain valuable insights into the quality of gametes, thereby optimizing fertility treatments for individuals and couples experiencing infertility issues. In this narrative review, we conducted a comprehensive search on PubMed, Google Scholar, and Scopus using the keywords "Machine Learning AND Quantification AND IVF". Eligible articles were initially screened based on their titles. After the title screening, a second screening was performed based on the abstracts of the selected articles. Finally, the full articles of the remaining studies were reviewed to ensure they met our inclusion criteria. From each eligible study, we extracted the following information: author(s) of the study, publication year, and the method employed to evaluate human oocyte quality. The development of a properly trained ML system will require careful attention to data quality, measurement, sample size, and ethics issues agreement.

Keywords: *Artificial intelligence, Deep learning, In vitro fertilization, Machine learning, Oocyte, Sperm.*

O-2

Comparison of pregnancy outcome after adding oral or intramuscular progesterone to vaginal progesterone in frozen embryo transfer: A cross-sectional study

Arabian S[1], Eftekhar M[1], Dashti S[1], Homayoon N[1], Nikfarjam E[1, 2].

1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Gynecology and Infertility Department, Shiraz Fertility Center, Shiraz, Iran.*

Email: eftekharmaryam1351@gmail.com

Background: Frozen embryo transfers (FET) now represent 41% of all embryo transfer cycles. While vaginal progesterone preparations have emerged as the preferred option for luteal phase support due to their ease of use, relying solely on vaginal progesterone in FET cycles has been associated with lower ongoing pregnancy rates. Intramuscular (IM) progesterone requires daily injections, which can be painful. Replacing IM progesterone with oral progesterone in women using vaginal progesterone for luteal phase support may result in comparable pregnancy outcomes.
Objective: This study aimed to evaluate the impact of replacing IM progesterone with oral dydrogesterone on pregnancy outcomes in FET cycles.
Materials and Methods: In this cross-sectional study, pregnancy outcomes were evaluated in women who underwent cleavage-stage FET during hormone replacement therapy endometrial preparation cycles at Yazd Reproductive Sciences Institute, Yazd, Iran, between April 2023 and November 2023. Participants were divided into 2 groups based on their LPS regimen: the dydrogesterone group, receiving vaginal progesterone combined with oral dydrogesterone, and the IM progesterone group, receiving vaginal progesterone alongside IM progesterone. Data were collected from participants records to compare pregnancy outcomes between groups.
Results: A total of 960 cycles meeting the inclusion criteria were analyzed, with 292 women in the dydrogesterone group and 668 women in the IM progesterone group, and pregnancy outcomes were compared between the 2 groups. The chemical pregnancy rates (28.4% vs. 29.9%, p = 0.636), clinical pregnancy rates (25.3% vs. 26.9%, p = 0.604), and ongoing pregnancy rates (21.9% vs. 23.8%, p = 0.525) were lower and miscarriage rates (14.7% vs. 11.7%, p = 0.210) were higher in dydrogesterone group compared to IM progesterone group, although this difference was not statistically significant.
Conclusion: Due to its convenient administration and similar pregnancy outcomes, oral dydrogesterone may serve as an effective alternative to daily IM progesterone injections.

Keywords: *Assisted reproductive technology, Luteal phase, Progesterone, Embryo transfer.*

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O-3

The impact of ovarian stimulation drugs on peripheral blood natural killer cells in women with endometriosis undergoing assisted reproductive technology

Balalak R[1], Elyasifar F[1], Azimi M[2], Takhiri F[3], Hafezi M[3], Amirchaghmaghi E[3], Ebrahimi M[3].
1.*Department of Cell and Molecular Biology Sciences, Faculty New Biological Sciences and Technologies, University of Science and Culture, ACECR, Tehran, Iran.*
2.*Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
3.*Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
Email: marzieh.ebrahimi@gmail.com

Background: Endometriosis, as an estrogen dependent disease in women of reproductive age could lead to infertility. Some infertile women with endometriosis undergo assisted reproductive technology (ART). On the other hand, it is suggested that natural killer (NK) cells, as one of the innate immune cells, play role in the pathogenesis of endometriosis. Although, different studies showed that long term use of gonadotropin-releasing hormone (GnRH) agonist drugs in the treatment of endometriosis could lead to alteration in NK cells percentage and activity in peripheral venous blood and peritoneal fluid. Still, the effect of short-term use of these hormonal drugs on NK cells in endometriosis is less studied.
Objective: This study aimed to evaluate the effect of ovarian stimulating drugs administered in long GnRH agonist and GnRH antagonist protocols on peripheral venous blood NK cells percentage and activity in infertile women with endometriosis undergoing ART.
Materials and Methods: In this prospective cohort study which was conducted at Royan Institute, Tehran, Iran between 2024 and 2025, 40 infertile women with endometriosis undergoing ART will be enrolled and follow up till ovum pick-up day. Venous blood samples from women with endometriosis were taken from participants before and after ovarian stimulation. Whole venous blood samples will be collected at 2 or 3 time points: 1) on the start day of the ovulation stimulation cycle (day 2-3 of the menstrual cycle), 2) on the day of starting gonadotropin (if possible) and 3) ovum pickup day. Each blood sample will be analyzed using specific antibodies against CD56 (NK cell surface marker), CD16 (another NK surface marker), CD3 (T cell marker) and CD107a (NK cell activity marker) by flow cytometry.
Results: Till now, 23 endometriosis women were enrolled. The mean body mass index was 24.72 Kg/m^2 and mean of age was 33.17 yr old. The mean percentage of NK cells (CD3-CD56 + cells) in starting day of stimulation cycle was 9.278 ± 0.9367% and in ovum pick up day was 9.201 ± 0.8514% which this difference was not statistically significant (p = 0.4719). The percentage of cytotoxicity markers (CD16+CD107a+) on NK cells was 8.746 ± 1.192% on the starting day of the stimulation cycle and 6.851 ± 0.9860% on the ovum pick-up day which its change was not significant (p = 0.1220).
Conclusion: Our preliminary data showed that the short-term use of GnRH agonist and antagonist drugs in ovarian stimulation protocols could not significantly change the percentage and cytotoxicity of venous blood NK cells. Completion of the sample size of the present study is needed for definite conclusion.
Keywords: *Endometriosis, Natural killer cells, Gonadotropin releasing hormone agonist, Gonadotropin releasing hormone antagonist, ART, Peripheral blood.*

O-4

Impact of metformin on reproductive outcomes after spermatogonial stem cell transplantation

Bashghareh A[1], Hedayatpour A[2], Modarresi P[3], Kazemzade Sh[4].
1.*Department of Anatomy, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.*
2.*Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
3.*Faculty of Veterinary Medicine, Islamic Azad University, Shabestar, Iran.*
4.*Shoushtar Faculty of Medical Sciences, Shoushtar, Iran.*
Email: alieh.bashghareh@yahoo.com

Background: Cryopreservation and transplantation of spermatogonial stem cells present a promising strategy for fertility restoration in patients who have undergone childhood cancer treatment. Additionally, metformin (MET), known for its antioxidant properties, may help mitigate chemotherapy-induced damage.
Objective: This experimental study aimed to assess the protective effect of MET against oxidative stress induced by busulfan and cadmium exposure, and to evaluate its impact on spermatogenesis restoration following spermatogonial stem cell transplantation (SSCT).
Materials and Methods: 40 adult male NMRI mice (6-8 wk) were used for the long-term infertility model induced by cadmium and busulfan treatment. The mice were divided into 4 groups (n = 10/each): busulfan and cadmium-treated, SSCT, MET-SSCT, and control. To evaluate the protective effect of MET against reactive oxygen species production, flow cytometry was employed. Proliferation and differentiation markers were assessed by immunofluorescence.

Results: Our findings demonstrated a significant reduction in reactive oxygen species production in the MET-treated cryopreservation group. Moreover, the expression of proliferation and differentiation markers following transplantation was significantly higher in the cryopreservation group with MET compared to the basic freezing medium group ($p \leq 0.001$).
Conclusion: Transplantation of spermatogonial stem cells with MET significantly enhances spermatogenesis and improves the homing efficiency of transplanted spermatogonial stem cells. This approach shows potential for fertility restoration in clinical settings, particularly for patients who have undergone childhood cancer treatments.
Keywords: *Azoospermia, Infertility, Metformin, Spermatogonial stem cells, Transplantation.*

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O-5
Designing an expert system to predict live birth in assisted reproductive technology cycles using machine learning

Farzandipour M[1], Esmaeili M[2], Tabibnejad N[3], Heydari SS[4].
1.*Health Information Management Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
2.*Department of Computer Engineering, Kashan Branch, Islamic Azad University, Kashan, Iran.*
3.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Department of Health Information Management and Technology, School of Allied Health Professions, Kashan, Iran.*
Email: safiyehsadat.heydari@gmail.com

Background: Infertility is defined as the inability to conceive naturally after one year of regular unprotected intercourse. Approximately one in six people worldwide experience infertility. The overall success rate of treatment using assisted reproductive technologies (ART) is low. Expert systems (ES) which designed based on mining the real data using machine learning can improve the clinical expert's decision-making and predicting the outcome of infertility treatments with greater accuracy and effectiveness. Association rule mining, as an algorithm in machine learning, can be used to identify relationships in data and designing ES.
Objective: This study aims to design an ES for predicting live birth rate (LBR) in ART cycles using association rule mining.
Materials and Methods: This research was done retrospectively. In the first stage, data on 4,290 cycles of infertile couples who referred to Yazd Reproductive Sciences Institute, Yazd, Iran from 2018-2020, which resulted in or did not result in LBR, were received in Excel. After data preprocessing, rules related to LBRwere extracted using Python. All extracted rules were evaluated based on reporting the indices of confidence, support, and experts opinions. The minimum support and the minimum confidence were considered 0.5 and 0.95, respectively. The web-based expert system was designed based on the selected final rules using JavaScript.
Results: 74 predictors were considered to extract association rules which resulted in 632 rules, of which 274 rules were evaluated calculating formal metrics including confidence and support. Finally, 32 rules were remained based on informal evaluation using experts opinions that were placed in the knowledge database of the ES. Then, ES was designed for six major causes of infertility including male infertility, unknown infertility, mixed infertility, polycystic ovary syndrome, premature ovarian failure, female factors except polycystic ovary syndrome and premature ovarian failure. 23 predictors were appeared in the antecedent (if) part of the final association rules.
Conclusion: The ES of LBR prediction can predict LBRin ART cycles. Considering the good performance of ES in predicting LBR, their use as an auxiliary tool in clinical decision-making is recommended.
Keywords: *Expert system, Association rules, Live birth, Machine learning, Infertility.*

O-6
A systematic review of assisted and third-party reproduction guidelines regarding management and care of donors

Iranifard E[1, 2], Ebrahimzadeh Zagami S[2, 3], Amirian M[4], Ebrahimipour H[5], Latifnejad Roudsari R[2, 3].
1.*Department of Midwifery, Maybod Branch, Islamic Azad University, Maybod, Iran.*
2.*Department of Midwifery, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran.*
3.*Nursing and Midwifery Care Research Center, Mashad University of Medical Sciences, Mashhad, Iran.*
4.*Department of Obstetrics and Gynecology, School of Medicine, Milad Infertility Treatment Center of Mashhad, University of Medical Sciences, Mashhad, Iran.*
5.*Department of Health Economics and Management, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.*
Email: rlatifnejad@yahoo.com

Background: Gamete and embryo donors encounter multifaceted challenges influencing their health and quality of life. Healthcare providers require comprehensive, evidence-based guidelines to support reproductive donors, effectively.
Objective: This systematic review aimed to synthesize current assisted and third-party reproduction guidelines regarding management and care of donors.
Materials and Methods: A systematic review was conducted, adhering to PRISMA guidelines. Databases including PubMed, Scopus, and Web of Science, as well as websites of relevant organizations including American Society for Reproductive Medicine, European Society of Human Reproduction and Embryology, and

Human Fertilization and Embryology Authority were searched without time restrictions up to July 2023. Search terms included "third-party reproduction", "gamete donation", "embryo donation", "guidelines", "committee opinion", and "best practice". Inclusion criteria encompassed English-language documents providing clinical or ethical guidance on donor management and care. Eleven guidelines were retrieved through related organizational. Websites and 317 studies were identified through searching electronic databases. After removing the duplicate studies and those articles that did not meet the inclusion criteria, full-texts of 21 articles were assessed for eligibility of which 16 articles were previous versions of included guidelines. Eventually, 5 studies that met the inclusion criteria and 9 guidelines were included in the review. The 14 documents, included 8 guidelines, 3 practice codes, and 3 committee opinions. Quality assessment was performed using the AGREE II instrument. The data of included documents were extracted and synthesized using narrative synthesis.

Results: The 14 documents including eight guidelines, 3 practice codes and three committee opinions originated from various regions across the world: 5 from the United States, 3 from Canada, 2 from the United Kingdom, 1 from Australia, 1 jointly from Australia and New Zealand, and 2 from the European Society of Human Reproduction and Embryology. Key areas addressed in these guidelines were classified into four categories including screening, counseling, information provision, and ethical considerations.

Conclusion: While existing guidelines offer fundamental recommendations for donors management and care, they often lack comprehensive coverage of critical areas affecting donors well-being. There is an urgent need for development of robust, evidence-based guidelines that holistically address the physical, psycho-social, ethical and legal needs of gamete and embryo donors. Future research should focus on these gaps to enhance donor care practices, globally.

Keywords: *Egg donors, Embryo donors, Sperm donors, Third party reproduction, Gamete donation, Guidelines, Systematic review.*

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O-7
Artificial intelligence-driven embryo selection in in vitro fertilization

Kalami Yazdi R[1], Mojavezi AR[1], Arjmand B[2].

1.*Iranian Cancer Control Center (MACSA), Tehran, Iran.*

2.*Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran.*

Email: barjmand@tums.ac.ir

The success of in vitro fertilization (IVF) largely depends on precise embryo selection for transfer. The standard procedure, traditional morphological evaluation by embryologists, is subjective and limited by inter-observer variability. This may result in less-than-ideal choices and reduced pregnancy rates. Artificial intelligence (AI), particularly deep learning algorithms, has unveiled a world of possibilities to improve embryo selection by objectively analyzing vast volumes of data combining genetic information and time-lapse images. The study's main objectives were to highlight the impact of AI on clinical results, compare it with conventional techniques, and explore the possibility of improving efficiency and standardization in IVF laboratories. Moreover, searching databases such as PubMed, Scopus, and Google Scholar by keywords such as "artificial intelligence", "embryo selection", "IVF", "deep learning", "time-lapse imaging", and "preimplantation genetic testing" revealed relevant results. In this context, the researchers suggest that AI systems analyze embryo images and time-lapse videos, in order to identify the features and patterns linked to implantation potential. AI-based selection often matched or exceeded the accuracy of traditional embryologist assessments, which provides greater objectivity, reduced variability, and the ability to process complex datasets. As a matter of fact, integration with time-lapse imaging enabled non-invasive monitoring, while some studies combined AI with genetic testing to refine selection. Furthermore, AI-driven embryo selection has the capacity to markedly enhance IVF outcomes by offering a more objective, consistent, and efficient approach to the identification of viable embryos. Although further extensive clinical studies are required to test its broad application and optimize AI algorithms, the present data suggests that AI has the capacity to transform embryo selection in IVF, thereby increasing pregnancy rates and creating better futures for patients. Future studies should focus on developing more complex AI models, combining several data sources (morphology, time-lapse, genetics), and addressing the ethical issues of AI application in clinical practice. The current situation of AI-driven embryo selection in IVF is investigated in this review, along with its possible advantages, constraints, and future possibilities.

Keywords: *AI, IVF, Infertility.*

O-8
Ameliorative effect of zinc oxide nanoparticles on daily sperm production and sperm parameters in mice under oxidative stress induced by taxol

Kahedi M, Mahmoodi M, Yengi Maleki Z.

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

Email: m-mahmoodi@araku.ac.ir

Background: Infertility is a significant issue for young men of reproductive age who are undergoing chemotherapy. Typically, chemotherapy drugs affect rapidly dividing germ cells, resulting in compromised spermatogenesis and a decrease in sperm count among cancer survivors.
Objective: This study investigated the effects of taxol on sperm parameters and oxidative stress and antioxidant properties of zinc oxide nanoparticles in adult male NMRI mice.
Materials and Methods: For doing this research at Arak University, Arak, Iran in 2023, 24 adult male NMRI mice (35 ± 2 gr, 8 wk) were divided into 4 groups (n = 6/each), control, taxol (5 mg/kg), zinc oxide nanoparticles (5 mg/kg) and taxol+zinc oxide nanoparticles groups. Animals were intraperitoneally treated for 35 days. Then, mice were anesthetized, and the caudal region of the left epididymis was used to measure the sperm parameters and the right testis to calculate the daily sperm production. Total antioxidant capacity and malondialdehyde levels were also measured and compared between groups.
Results: Infertility is a significant issue for young men of reproductive age who are undergoing chemotherapy. Typically, chemotherapy drugs affect rapidly dividing germ cells, resulting in compromised spermatogenesis and a decrease in sperm count among cancer survivors.
Conclusion: The results of this research indicated that zinc oxide nanoparticles, because of their strong and effective antioxidant characteristics, have the ability to mitigate the harmful impacts of taxol on sperm quality.
Keywords: *Taxol, Zinc oxide nanoparticles, Sperm, Oxidative stress, Mice.*

O-9
The effect of exosomes extracted from human embryonic stem cells derived mesenchymal stem/stromal cells on polycystic ovary syndrome in rat model

Karimi M[1], Aflatoonian B[1, 2, 3].
1.*Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: b.aflatoonian@ssu.ac.ir

Background: Polycystic ovary syndrome (PCOS) is the most medically cause of female infertility, which several treatment methods have been used to treat this syndrome. However, recently stem cell therapy opened new hopes for the patients and clinicians to treat PCOS more effective in a safer approach. The secretome of these cells contains growth factors, cytokines and extracellular vesicles (exosomes, micro-vesicles and apoptotic bodies), which are effective in the treatment of PCOS.
Objective: We aimed to investigate the effect of exosomes extracted from human embryonic stem cells derived mesenchymal stem/stromal cells on PCOS in rat.
Materials and Methods: 24 adult female Wistar rats were randomly separate into 4 groups of control (C), PCOS, treatment 1 (T1), and treatment 2 (T2). After induction of PCOS using letrozole (1 mg/kg) dissolved in normal saline (2 ml/kg), in T1 and T2 groups they were treated with intra ovarian injection of exosomes (50 and 100 µg/µl, respectively). At the end of the treatment period, the blood testosterone level, and weight and size of ovaries were measured and compared between groups.
Results: The weight ($p = 0.017$) and size ($p = 0.019$) of ovaries significantly increased in the PCOS group compared to C group, and significantly decreased in T1 and T2 groups. Also, testosterone level in T1 and T2 groups were significantly lower than the PCOS group ($p = 0.018$). In sum in T2 group exosome treatment was significantly more efficient than T1 group.
Conclusion: Our findings indicated that exosomes extracted from human embryonic stem cells derived mesenchymal stem/stromal cells improve PCOS treatment in animal model especially in higher concentration (100 µg/µl).
Keywords: *Extracellular vesicles, Exosomes, Human embryonic stem cells, Mesenchymal stem/stromal cells, Polycystic ovary syndrome.*

O-10
Impact of Vitamin D3 Supplementation on Sperm Quality, Oxidative Stress Markers, and Reproductive Hormones in Infertile Men with Asthenospermia: A Triple-Blind Clinical Trial

Maghsoumi-Norouzabad L[1], Zare Javid A[2], Moini Jazani A[1], Nasimi Doost Azgomi R[1].
1.*Traditional Medicine and Hydrotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.*
2.*Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
Email: L.maghsumi55@gmail.com

Background: The male reproductive system is known to be a target tissue for vitamin D3 (VD3). Animal models and cross-sectional cohort studies have suggested a beneficial role for VD3 in male reproduction.
Objective: This study aimed to evaluate the effects of VD3 supplementation on sperm parameters, seminal and serum levels of oxidative stress, and serum endocrine factors in asthenospermia infertile men.
Materials and Methods: This randomized, triple-blind, placebo-controlled clinical trial was conducted on 86 asthenospermia infertile men with serum 25-hydroxy VD3 (25-OH-D3) < 30 ng/ml who were referred to the infertility clinic of Ahvaz Jahad Daneshgahi, Ahvaz, Iran, from 2018-2020. Participants were randomly allocated by statistical software using classified

randomized blocking method (4 blocks) based on age (20-40 and 40-49) and sperm concentration (5-15 and 15-20 million/ml) to groups A and B, who received 4000 IU VD3 daily and a matching placebo for 3 months. Demographic data, dietary intake, physical activity, sun exposure, anthropometric indices, serum and seminal levels of malondialdehyde, 8-hydroxy-2-deoxy guanosine, total antioxidant capacity and calcium, sperm DNA fragmentation index, serum 25-OH-D3, luteinizing hormone, follicle-stimulating hormone, total testosterone, estradiol, sex hormone-binding globulin, total testosterone/luteinizing hormone and total testosterone/estradiol ratios, prolactin, parathyroid hormone, osteocalcin, phosphorus and sperm parameters including semen volume, total sperm count, sperm motility and sperm morphology were assessed before and after intervention.

Results: VD3 supplementation led to a significant increase in serum 25-OH-D3, phosphorus, serum and seminal calcium, total antioxidant capacity, the total testosterone/luteinizing hormone ratio, and both total and progressive sperm motility. Furthermore, it caused a notable decrease in serum and seminal malondialdehyde as well as serum parathyroid hormone in comparison to the baseline and placebo groups. However, it did not produce significant effects on body weight, body mass index, waist circumference, body fat, 8-hydroxy-2-deoxyguanosine, DNA fragmentation index, serum osteocalcin, luteinizing hormone, follicle-stimulating hormone, total testosterone, estradiol, sex hormone-binding globulin, prolactin, total testosterone/estradiol ratio, semen volume, sperm count, and normal sperm morphology.

Conclusion: VD3 effectively improves infertility in men with idiopathic asthenospermia with serum 25-OH-D3 < 30 ng/ml by reducing oxidative stress and increasing total and progressive sperm motility.

Keywords: *Vitamin D3, Oxidative stress, Idiopathic asthenospermia, Sperm quality, Sex hormones.*

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Maghsoumi-Norouzabad L, Zare Javid A, Mansoori A, Dadfar M, Serajian A. The effects of vitamin D3 supplementation on spermatogram and endocrine factors in asthenozoospermia infertile men: A randomized, triple blind, placebo-controlled clinical trial. *Reprod Biol Endocrinol* 2021; 19: 102. Doi: 10.1186/s12958-021-00789-y.

O-11

Impact of pentoxifylline treatment on embryo morphokinetic using a time-lapse imaging system: A sibling oocyte study

Mahaldashtian M[1], Khalili MA[1], Agharahimi A[1], Mangoli E[1], Palmerini MG[2], Nottola SA[3], Macchiarelli G[3], Woodward B[4].

1.*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

2.*Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy.*

3.*Department of Human Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome, Rome, Italy.*

4.*X&Y Fertility, Leicester, UK.*

Email: khalili59@hotmail.com

Background: Pentoxifylline is one of the motility enhances in Andrology field that can be used for increase sperm motility in azoospermic samples.

Objective: The aim of this research was to evaluate the influence of pentoxifylline (PTX) on in vitro embryo morphokinetics and clinical results by means of non-invasive time lapse monitoring system.

Materials and Methods: This prospective non-blind sibling oocyte study comprised a total of 57 non-obstructive azoospermia men, with a mean age of 36.38 ± 6.99. This study was done at Yazd Reproductive Sciences Institute, Yazd, Iran from April 2021 to 2022, approved. All individuals gave informed consent to participate in the study. Inclusion criteria were NOA peoples younger than 50 yr with female partners less than 40 yr old having at least 4 mature oocytes, with no infertility problems. Exclusion criteria included HIV/HPV positive peoples, oocyte donation cycles and cycles with missing key data. Half of the mature oocytes from each individual were injected with PTX-treated sperm (3.6 Mm PTX, 30 min, 37C) as PTX group and the remaining half was injected with non-treated sperm as control group. Embryo morphokinetics were evaluated by means of time lapse monitoring system. Fertilization was assessed 16 hr after insemination, and embryo transfer was done on day 3 post-injection. Chemical and clinical pregnancies were assessed 2, and 7 wk after embryo transfer, respectively.

Results: In the control group, 250 metaphase II oocytes were injected, 127 oocytes were fertilized, and 111 embryos were formed. In the PTX group, however, 247 metaphase II oocytes were injected, 158 oocytes were fertilized, and total of 147 embryos were formed. There were significant differences in the number of 2 pronucleus formation, fertilization rate and embryo formation between the groups ($p < 0.05$). Embryos from the PTX group had a significantly faster rate for time pronucleus fading and time to reach the 2- and 4-cell stages ($p < 0.05$). The results of pregnancies were similar between the groups.

Conclusion: PTX affect embryo morphokinetic and increased the speed of cell divisions without any adverse effects on cleavage patterns and pregnancy outcome.

Keywords: *Pentoxifylline, Morphokinetics, Non-obstructive azoospermia, Time-lapse imaging.*

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O-12

Assesment the cryopreservation effect of pentoxifylline on premeiotic and postmeiotic marksers after transplantation of frozen-thawed spermatogonial stem cell to azoospermic mouse model caused by torsion

Malekzadeh M, Homayoun M.

Department of Anatomy, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Email: mehrnoush.malekzadeh@med.mui.ac.ir

Background: Spermatogonial stem cells (SSCs) play a pivotal role in sustaining spermatogenesis and male fertility. Cryopreservation of SSCs is essential for fertility preservation, yet optimizing protocols to maintain their functionality post-thawing remains a challenge. This study investigates the cryoprotective potential of pentoxifylline (PTX) in preserving SSC viability and differentiation capacity after transplantation into an azoospermic mouse model.

Objective: To evaluate the effects of PTX supplementation in cryopreservation media on the proliferation and differentiation of SSCs following freeze-thaw cycles and transplantation.

Materials and Methods: This was an experimental study using an in vivo azoospermia mouse model to evaluate the effects of PTX on cryopreserved SSCs post-transplantation. SSCs were isolated purity was confirmed by surface markers (α6-integrin, β1-integrin, ID4) and divided into 4 experimental groups (n = 6/each): fresh SSCs, fresh SSCs treated with PTX, SSCs cryopreserved in basal medium, and SSCs cryopreserved with 10 mM PTX. These cells were transplanted into azoospermic mice induced by testicular torsion. After eight weeks, recipient testes were analyzed for SSC proliferation (via MVH and ID4 markers) and differentiation (using c-Kit, SCP3, Tnp1, Tnp2, and Prm1 markers) by immunohistochemistry and Western blot and real-time polymerase chain reaction.

Results: Flow cytometry and morphological assessments confirmed successful SSC enrichment, evidenced by colony-forming ability and expression of ID4, α6-integrin, and β1-integrin. PTX-treated cryopreserved SSCs exhibited superior proliferative (Ddx4 and C-Kit proteins) and differentiative (ID4 and Sycp3 Protein) potential compared to cryopreservation group ($p < 0.0001$). There was a significant increase in the expression of *Tnp1*, *Tnp2*, and *Prm1* genes in the cryopreservation group with PTX compared to the cryopreservation group ($p < 0.0001$). Transplanted SSCs colonized seminiferous tubules and restored spermatogenesis, yielding mature sperm.

Conclusion: The addition of 10 mM PTX to cryopreservation media significantly enhances SSC recovery and functionality post-thawing, supporting its potential use in fertility restoration. Further studies are warranted to validate long-term safety and clinical applicability.

Keywords: *Male infertility, Cryopreservation, Spermatogonial stem cells, Transplantation, Antioxidant, Pentoxifylline.*

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O-13

Apoptotic genes expression for assessment of radioprotective effect of omeprazole against testicular damage induced by ionizing radiation in mice

Ranjbar Fakhrabadi S[1], Javid A[1], Montazeri F[2], Biabani-Ardakani J[3], Rezaei-Pandari M[3].

1.*Department of Biological Sciences, Faculty of Engineering and Science, Science and Arts University, Yazd, Iran.*

2.*Abortion Research Centre, Yazd Reproductive Sciences Institute, Yazd, Iran.*

3.*Biotechnology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: javadbiabani@ssu.ac.ir

Background: Since the discovery of cysteine's radioprotective effect in 1949, extensive research has sought other radioprotective compounds. Evaluating drugs with known pharmacokinetics offers a quick path to clinical solutions. Omeprazole's antioxidant and anti-inflammatory activities suggest its potential radioprotective effects. Similar compounds, such as atorvastatin and L-carnitine, have shown efficacy in reducing oxidative stress and inflammation.

Objective: This study aims to evaluate the radioprotective effects of omeprazole on testicular damage induced by ionizing radiation in mice by assessing apoptotic gene expression (*BAX*, *BCl2*).

Materials and Methods: In this experimental study, 36 adult male Syrian mice (6-8 wk old, 30-35 gr) were randomly divided into 6 groups (n= 6/each) and acclimatized for 1 wk under standard conditions (12-hr light/dark cycles, 26-28°C, 50 ± 10% humidity). The groups were: group 1 (control): mice received 0.5% carboxymethyl cellulose containing 0.9% sodium chloride. Group 2 (radiation): mice were only exposed to total body radiation with a single dose of 6 Gy with a LINAC-X-ray accelerator. Group 3 (omeprazole [30 mg/kg]): mice received 30 mg/kg daily via gavage for 7 days. Group 4 (omeprazole [50 mg/kg]): mice received 50 mg/kg daily via gavage for 7 days. Group 5 (radiation + omeprazole [30 mg/kg]): mice received 30 mg/kg omeprazole daily via gavage for 7 days before 6 Gy whole-body irradiation. Group 6 (radiation + omeprazole [50 mg/kg]): mice received 50 mg/kg

omeprazole daily via gavage for 7 days before 6 Gy whole-body irradiation. Body weights were measured at the start and end of the study. On day 8 (one day after the final dose), groups 2, 5, and 6 were irradiated and sacrificed on day 9 of the study (n = 3/each) and on day 15 of the study (n = 3/each). The tissues samples were collected post-euthanasia and preserved in RNA later solution, subsequently stored at -80°C. Total RNA extraction was achieved using the TRIzol reagent. Post-extraction, complementary DNA synthesis was performed to conduct the subsequent quantitative polymerase chain reaction analysis. Real-time quantitative polymerase chain reaction was employed to analyze the expression levels of specific genes associated with apoptosis including *BAX* and *BCl2*. At the same time, GAPDH served as an internal control for normalization.

Results: Radiation significantly increased *BAX* and decreased *BCl2* gene expression, indicating enhanced apoptosis. Omeprazole treatment, especially at 50 mg/kg, mitigated radiation-induced changes in gene expression. The combination of radiation and omeprazole resulted in lower (higher) *BAX* (*BCl2*) expressions compared to radiation alone, suggesting potential protective effects.

Conclusion: Omeprazole shows promise as a radioprotective agent, notably in reducing radiation-induced apoptosis. Higher doses of omeprazole (50 mg/kg) were more effective in mitigating adverse gene expression changes caused by radiation. These findings support further investigation of omeprazole for clinical radioprotection applications.

Keywords: *Omeprazole, Apoptosis, Gene expression, Radiation protection.*

O-14
Advancing in vitro spermatogenesis: The role of hyaluronic acid-alginate and soft agarose in testicular tissue engineering

Rashki Ghaleno L[1], Shahverdi A[1], Rezazadeh Valojerdi M[1, 2].

1. *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

Email: mr_valojerdi@yahoo.com

Background: Cancer treatments for prepubertal boys, such as testicular radiation therapy and chemotherapy, carry a significant risk of adversely impacting fertility.

Objective: This study involved the culture of testicular cells and tissue utilizing natural composites in both 2-dimensional and 3-dimensional (3D) formats, thereby enhancing both methodologies.

Materials and Methods: This experimental study was conducted in 2 phases in Royan institute, Tehran Iran. First, biomaterials containing 0.5% hyaluronic acid, 1% alginate, 2.5 mg/ml collagen, and produced 25 mg/ml decellularized extracellular matrix from six Ram testicles were utilized to create composites. hyaluronic acid-alginate (HA-Alg) was chosen as a superior composite after 14 days of 3D culture of 5 days post-partum (dpp) mouse testicular cells because of the larger quantity and size of the organoids that were formed. Subsequently, cell culture was conducted using HA-Alg for 14 days, which was later prolonged by an additional 28 days. Morphology and gene expression were analyzed employing suitable techniques. During the second phase, testicular tissues from neonate mice were positioned on 1.5% agarose, following conventional agarose (C.AG) methods, alongside soft hydrogel layers in a soft agarose culture system, which comprised 0.35% agarose (S.AG), 1% alginate (S.AL), and a combination of 0.5% hyaluronic acid and 1% alginate (S.H-AL). This research examined histological aspects, and assessed the expression of genes associated with spermatogenesis.

Results: On day 14, histology and immunostaining tests demonstrated the presence of hepatocyte-like cells (HLCs) and albumin production, indicating HLC functionality. The gene expression confirmed the expression of angiogenesis markers ($p = 0.006$). Following a 28-day culture period, testicular cells at 5 dpp differentiated into erythrocytes and hepatic-like cells, while a limited number of organoids exhibited characteristics of renal cells. In the second phase, on day 28, the total number of tubules in the C.AG and S.AG groups was significantly greater than in the S.H-AL group ($p = 0.003$). The interstitial area demonstrated a notable increase from day 5-28 across all groups ($p = 0.021$), with S.AG and S.AL presenting larger areas than C.AG ($p = 0.045$). Histological evaluation of cultured tissues demonstrated a significantly greater presence of spermatogonia, spermatocytes, and round spermatids in the S.AG group relative to the other groups ($p = 0.038$) In contrast, the S.AL group predominantly exhibited tubules containing only Sertoli cells. Gene expression data revealed significantly elevated levels of synaptonemal complex protein 3 and transition protein 1 in the S.AG group relative to the C.AG and S.AL groups ($p = 0.008$).

Conclusion: The HA-Alg composite did not facilitate spermatogenesis in the 3D culture of mouse testicular cells; however, it exhibited an unexpected capacity to promote the differentiation of neonate mouse testicular cells into HLC, erythrocytes, and various other cell lineages. The second stage confirmed the effectiveness of S.AG for organ culture of testis tissues and enhanced in vitro spermatogenesis compared to C.AG and promotes the differentiation of SSC into round spermatids.

Keywords: *Spermatogenesis, Organoids, Cell culture, Tissue culture.*

O-15
Assessing the needs of sexual health and designing an educational package for women with breast cancer in reproductive age

Seyyedzadeh N, Moghaddam-Banaem L.
Department of Reproductive Health and Midwifery, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
Email: moghaddamb@modares.ac.ir

Background: Breast cancer is the most common cancer in women, with 1.38 million new cases yearly. It accounts for 30% of new cancers and ranks second in cancer-related deaths. Iranian women face earlier onset, about a decade sooner than in developed countries. While treatments improve survival, they often cause lasting physical and psychological effects, including sexual health challenges, underscoring the need for specialized education for survivors.
Objective: The present study was designed to develop a sexual health education package, with impact on sexual quality of life, sexual function, and marital satisfaction in the survivors of breast cancer.
Materials and Methods: This mixed-method study was conducted in Tehran, in 2022 in 2 phases: a qualitative study with 18 participants, a semi-experimental study with 37 participants. In the first phase a qualitative study was performed with directed content analysis, based on Woods' Gendering theory, then a sexual health education booklet and an educational package for breast cancer survivors were developed. The second phase involved the implementation of the sexual health education package in a single group of 37 breast cancer survivors, conducted over three weeks, with sessions twice a week virtually, through the WhatsApp social media platform for individual participants. The study outcomes measured online before, immediately after, and three months after the intervention, were the components of three questionnaires: the female sexual quality of life questionnaire, the female sexual function index, and the Enrich marital satisfaction scale (35 questions).
Results: At the conclusion of the qualitative phase, a matrix table for sexual health was developed for breast cancer survivors using Woods' Gendering theory, identifying 3 main themes: sexual self-concept, sexual function, and sexual relations. The analysis resulted in 11 categories, 31 sub-categories, 78 axial codes, and 285 initial codes. An educational booklet for survivors and a guide for educators were produced. An electronic content was also prepared. In the semi-experimental phase, significant improvements were observed in sexual quality of life immediately, and 3 months after intervention ($p = 0.028$, and 0.036 respectively), sexual desire ($p = 0.041$), and satisfaction ($p = 0.026$), and ideal distortion immediately, and 3 months after intervention ($p = 0.009$, and 0.008 respectively). Changes in other areas were not significant.
Conclusion: In the present study a sexual health education package for breast cancer survivors was developed and implemented, which had a statistically significant impact on sexual quality of life, sexual function, and marital satisfaction; however, the clinical significance of these effects are still unknown. Thus, further combined or multifaceted interventions aimed at comprehensive sexual health improvement in these women seem necessary.
Keywords: *Sexual health, Education, Breast cancer, Reproductive age.*

O-16
Adipose mesenchymal stem cell conditioned medium enhances ovarian function in rats with polycystic ovary syndrome: A focus on epigenetic modifiers

Shafiei G[1], Saheli M[2], Ganjalikhan-hakemi S[2], Haghpanah T[2], Nematollahi-Mahani SN[2, 3].
1.*Anatomical Sciences Research Center, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran.*
2.*Anatomical Sciences Department, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.*
3.*Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*
Email: thaghpanah1984@gmail.com; nnematollahi@kmu.ac.ir

Background: Polycystic ovary syndrome (PCOS) is a multifaceted condition characterized by a range of epigenetic influences. Recent studies have highlighted the regenerative potential of adipose-derived mesenchymal stem cells (ASCs) and their conditioned medium (ASC-CM) in promoting tissue repair.
Objective: The present study aimed to evaluate the effects of ASCs and ASC-CM on epigenetic regulatory mechanisms, steroidogenic activity, and folliculogenesis in a rat model of PCOS induced by letrozole.
Materials and Methods: In this experimental study, 60 adult female Wistar rats (8 wk, 200 ± 20 gr) were randomly assigned to receive either 1% carboxymethyl cellulose (CMC group, n = 10) and 1 mg/kg letrozole orally for 21 days (PCOS group, n = 50). On day 22, the PCOS group was divided into four subgroups (n = 10/each): 1) ASC group, receiving 2×10^6 ASCs suspended in 20 µl culture medium; 2) ASC-CM group, treated with 20 µl of ASC-CM; 3) control (CTRL) group, receiving 20 µl of culture medium; and 4) sham group, treated with 20 µl of normal saline. All transplantations were occurred on 22th day and were performed via direct injection into the ovaries. 4 wk post-treatment, an oral glucose tolerance test was conducted to confirm the PCOS model, and folliculogenesis was evaluated alongside the gene

expression of DNA methyltransferases (DNMT1, 3A, and 3B), as well as histone deacetylases (HDAC1 and HDAC2) using real-time polymerase chain reaction. The expression of estrogen receptors α and β in the ovaries was assessed through real-time polymerase chain reaction and Western blotting, and serum estradiol levels were measured. Additionally, the levels of 5-methyl-cytosine and 5-hydroxymethyl-cytosine were determined by immunohistochemistry. Physical parameters, including body weight and estrous cycle patterns, were recorded.

Results: PCOS-induced rats in the sham and CTRL groups exhibited reproductive dysfunctions, characterized by irregular estrous cycles, increased ovarian dimensions, elevated body weight, and altered blood glucose levels. These abnormalities were significantly improved, particularly in the ASC-CM group. ASC-CM treatment was more effective than ASC in enhancing the expression of DNMT1, 3A, and 3B ($p = 0.050$), as well as HDAC1 ($p = 0.006$) and HDAC2 ($p = 0.050$) genes and estrogen receptor α and β at both the gene and protein levels. Serum estradiol levels and the amount of 5-methyl-cytosine were significantly increased in the ASC-CM group. Furthermore, a notable reduction in the number of primary and preantral follicles was observed in the ASC-CM group compared to the sham ($p < 0.0001$), and CTRL ($p = 0.000$) groups.

Conclusion: Treatment of PCOS rats with ASC and ASC-CM can improve folliculogenesis, steroidal secretion and function. ASC-CM administration was more effective than ASC administration.

Keywords: *Adipose-derived mesenchymal stem cells, DNA methylation, Histone deacetylases, Estrogen receptor, Polycystic ovary syndrome.*

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O-17

Evaluation of fibroblast growth factor (FGF) 1, *FGF2*, and *FGF13* gene expression following endometrial scratching in women with unexplained repeated implantation failure: A randomized controlled trial

Aghajanpour S[1], Hosseini E[2], Zandieh Z[3], Amjadi FS[3], Aflatoonian R[1].

1. *Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.*
2. *Department of Obstetrics and Gynecology, Mousavi Hospital, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.*
3. *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

Email: Amjadi.bh@gmail.com; r.aflatoonian@gmail.com

Background: Angiogenesis plays a vital role in implantation, decidualization, and placentation. Fibroblast growth factors (*FGFs*) significantly impact cellular processes, including angiogenesis. Inadequate angiogenesis during the implantation window may impair endometrial receptivity, leading to unexplained repeated implantation failure (uRIF). Endometrial scratching (ES) has been suggested as a potential method to enhance implantation rates in uRIF. This study investigated the expression of fibroblast growth factor 1 (*FGF1*), *FGF2*, and *FGF13* genes, which contribute to endometrial angiogenesis, following ES.

Objective: Due to poor vascularization in women with uRIF, this study investigated the endometrial angiogenic response to ES by evaluating *FGF1*, *FGF2*, and *FGF13* gene expression.

Materials and Methods: This study received ethical approval from Iran University of Medical Sciences, Tehran, Iran and endometrial tissue samples were collected from women at Laleh hospital in Tehran, Iran, between June 2021 and January 2023. In this double-blind randomized clinical trial, 20 infertile women with uRIF were divided into 2 groups: one underwent ES in the follicular phase, while the other did not. Gene expression of *FGF1*, *FGF2*, and *FGF13* in secretory-phase endometrial biopsy samples was analyzed via quantitative polymerase chain reaction.

Results: The findings of this study revealed a significant increase in the relative expression of *FGF1* ($p = 0.001$), *FGF2* ($p = 0.041$), and *FGF13* ($p = 0.003$) in the intervention group following ES, compared to the control group.

Conclusion: *FGF1*, *FGF2*, and *FGF13* are members of the *FGFs* family, which plays a crucial role in angiogenesis during the window of implantation, helping to prepare the endometrium for embryo implantation. On the other hand, it has been demonstrated that the reduction of these genes leads to insufficient angiogenesis, potentially resulting in failure of embryo implantation. It has been determined that embryo implantation does not occur in RIF women, possibly due to inadequate angiogenesis. The results of the present study indicated that ES induced significant alterations in the expression of the *FGF1*, *FGF2*, and *FGF13* genes and, as a result, may have improved endometrial angiogenesis.

Keywords: *Angiogenesis, Fibroblast growth factor, Endometrium.*

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O-18

The role of leukemia inhibitory factor on expression of genes related to angiogenesis in recurrent abortion: A mice model study

Allahyari Z[1], Zare F[2], Nikoonahad N[3].

1. *Department of Molecular Genetics, Faculty of Sciences, Science and Arts University, Yazd, Iran.*
2. *Reproducive Immunology Research Center, Shahid Sadoudghi University of Medical Sciences, Yazd, Iran.*

3.*Biology Department, Faculty of Sciences, Science and Arts University Yazd, Iran.*
Email: z.allahyari4450@gmail.com

Background: Recurrent miscarriage (RM) is a primary pregnancy complication that affects approximately 1-3% of pregnancies. Multiple factors contribute to RM. Leukemia inhibitory factor (LIF), a cytokine belonging to the interleukin-6 family, plays a critical role in various biological processes, particularly during pregnancy and fetal development.
Objective: This study aimed to investigate the role of LIF on the expression of angiogenesis-related genes - fibroblast growth factor (*FGF*), angiopoietin-1 (*ANG1*), platelet derived growth factor (*PDGF*), transforming growth factor-beta (*TGF-β*) and vascular endothelial growth factor (*VEGF*)- in a mouse model of RM.
Materials and Methods: Female CBA/J mice mated with DBA/2J males were used to establish a RM model. The mice were randomly divided into 3 groups: normal group: CBA/J females mated with Balb/c males without any injection. RM control group: CBA/J × DBA/2J mice receiving a PBS injection, and LIF-treated RM group: CBA/J × DBA/2J mice injected with LIF. After confirming pregnancy by the presence of a vaginal plug, mice were sacrificed on days 4, 7, and 14 of gestation. Uterine and placental tissues were collected for analysis of angiogenesis-related gene expression (*VEGF*, *PDGF*, *ANG1*, *FGF*, and *TGF-β*) using real-time polymerase chain reaction.
Results: On gestational days 4 and 7, no significant differences in gene expression were observed between the groups. However, by day 14, the expression of all target genes was increased in the LIF-treated RM group compared to the other groups. This increase was statistically significant for *VEGF* and *TGF-β* ($p = 0.03$ and $p = 0.04$, respectively). A similar pattern was observed in placental tissues, with higher expression levels in the LIF-treated group, although these changes were not statistically significant. Correlation analysis between uterine and placental gene expression on day 14 showed no significant associations.
Conclusion: The findings suggest that LIF administration in an RM mouse model enhances the expression of angiogenic factors, particularly on day 14 of pregnancy. The significant upregulation of key genes such as *VEGF* implies a potential role for LIF in supporting fetal development by promoting angiogenesis. These results reinforce the known importance of LIF in oocyte maturation, embryo implantation, and embryonic development.
Keywords: *Leukemia inhibitory factor, Recurrent miscarriage, Vascular endothelial growth factor.*

O-19
Stemness properties of amniotic fluid mesenchymal stem cells during proliferative senescence

Azad FS[1, 2], Zare E[2, 3], Hoseini SM[4], Montazeri F[2].
1.*Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
4.*Biotechnology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: marjan.montazeri@gmail.com

Background: Amniotic fluid-derived mesenchymal stem cells (AF-MSCs) have garnered significant attention in regenerative medicine due to their unique properties, such as multilineage differentiation potential and self-renewal ability. AF-MSCs are susceptible to senescence, a biological process characterized by a decline in cell division and function. Stemness attributes, such as self-renewal capacity and differentiation potential, are critical for the efficacy of MSCs in the regeneration. As AF-MSCs undergo senescence, there is often a measurable decrease in these properties, influencing their overall functionality in clinical applications. MYB proto-oncogene protein *2 (B-MYB or MYBL2*) is known as one of the genes involved in senescence, and its relation with some stemness genes such as Thy-1 cell surface antigen *(Thy-1*), Mast/Stem cell growth factor receptor *(C-Kit*), and fibroblast growth factor *(FGF*) were investigated.
Objective: The main goal of this study was to evaluate the expression level of the *B-MYB* gene as a marker of senescence in AF-MSCs compared to stemness genes in different phases of growth.
Materials and Methods: This in vitro experimental study aimed to evaluate senescence and stemness-related markers in AF-MSCs. AF-MSCs were isolated from seven pregnant women via amniocentesis at the Yazd Reproductive Sciences Institute, Yazd, Iran. Homogeneity was confirmed by flow cytometry. Senescence was assessed using beta-galactosidase activity through X-gal staining.
Results: The *B-MYB* gene as a marker of senescence exhibited up-regulation in senescent AF-MSCs compared to those in the proliferative stage. Additionally, molecular analysis revealed a significant increase in the expression levels of genes as a marker of stemness (*Thy-1, C-Kit*, and *FGF*) during senescence. Expression levels of stemness-related genes *Thy-1* ($p = 0.0135$), *C-Kit* ($p = 0.0351$), and *FGF* ($p = 0.0397$) were analyzed. Total RNA was extracted using TRIzol, cDNA was synthesized using the first strand cDNA synthesis kit (Thermo Scientific), and quantitative polymerase chain reaction was performed with *GAPDH* as the reference gene.

Conclusion: This study reveals that senescence in AF-MSCs is accompanied by increased expression of *B-MYB* and key stemness-related genes such as *Thy-1*, *C-Kit*, and *FGF*. These findings suggest that senescence does not necessarily lead to a loss of stemness and may even preserve or enhance certain stem cell traits. Targeting the interplay between senescence and stemness could improve the therapeutic potential of AF-MSCs in regenerative medicine. Further research is needed to clarify the mechanisms involved.

Key words: *Mesenchymal stem cells, Amniotic fluid, Cellular senescence, B-MYB gene expression, Stemness genes.*

O-20
Evaluation of *NLRP3* and *Caspase-1* gene expression in endometrial tissue obtained during the window of implantation in women with secondary infertility and isthmocele

Azadegan M[1], Ahani Nahayati D[2], Jamshidian F[1], Aghajanpour S[3], Aflatoonian R[3], Hafezi M[3].

1.*Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran.*
2.*Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, 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: azadmobin96@gmail.com; maryamhafezi90@yahoo.com

Background: Embryo implantation failure is a significant challenge for infertility patients, influenced by multiple factors, including immune cell activity and inflammation. Endometrial immune cells strongly express the *NLRP3* inflammasome, which regulates *Caspase-1* activation and pro-inflammatory cytokine secretion (*IL-1β*, *IL-18*). Excessive activation of this pathway may disrupt endometrial receptivity, leading to infertility. Chronic inflammation caused by blood accumulation in the cesarean scar area could be one of the possible factors contributing to a decreased implantation rate due to isthmocele. Therefore, isthmocele, a myometrial scar from cesarean sections, is frequently linked to secondary infertility. While its exact role in subfertility remains unclear, it likely affects embryo implantation and reduces fertility.

Objective: Due to the potential presence of inflammation at the cesarean scar site, this study examined the expression levels of *NLRP3* and *Caspase-1* genes in the endometrium of women with isthmocele.

Materials and Methods: This study received ethical approval from the Royan Institute Committee, Tehran, Iran and endometrial tissue sampling was conducted between 2023 and 2024. In this case-control study, 10 infertile women with isthmocele grade 3 (case group) and 10 fertile women (control group) participated. Endometrial biopsy during the window of implantation was done for both groups. *NLRP3* and *Caspase-1* mRNA gene expression were assessed using quantitative polymerase chain reaction.

Results: Using quantitative polymerase chain reaction, it was shown that *NLRP3* and *Caspase-1* ($p \leq 0.051$) were expressed differently in the endometrium of the case group compared to the control group.

Conclusion: Therefore, isthmocele may be associated with uterine inflammation because of the excessive expression of the *NLRP3* inflammasome (*NLRP3* and *Caspase-1*); hence, it can create an unfavourable environment for embryonic implantation.

Keywords: *NLRP3 inflammasome, Infertility, Endometrium.*

O-21
Impact of abnormal semen parameters on total oxidative status and DNA/chromatin integrity

Imani M[1], Vahedi Raad M[2], Afsari M[1], Fesahat F[1].

1.*Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Department of Anatomical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.*

Email: farzaneh.fesahat@gmail.com

Background: Male infertility is a significant health concern, impacting approximately 7% of the male population worldwide. Among various contributing factors, oxidative stress has emerged as a key element in the pathogenesis of impaired fertility. It can influence sperm function by damaging cellular structures, including DNA and chromatin, thereby compromising fertilizing capacity.

Objective: This study aimed to compare the total antioxidant status and DNA/chromatin integrity in semen samples from infertile men with different abnormal semen profiles against fertile men with normal semen parameters.

Materials and Methods: In this cross-sectional analysis, semen samples were obtained from 81 men and categorized into five groups: normozoospermic fertile controls (n = 22) and four infertile subgroups: teratozoospermic (n = 15), asthenoteratozoospermic (n = 13), oligoasthenoteratozoospermic (n = 13), and azoospermic (n = 18). Seminal plasma total antioxidant capacity and total oxidant status were measured using enzyme-linked immunosorbent assay methods. Chromatin and DNA integrity were assessed via chromomycin A3 and toluidine blue staining. All participants were < 40 yr of age and had a body mass index of ≤ 25. Control group participants had normal semen parameters, at least one child in the past 2 yr, no smoking, no varicocele, no medication use. Experimental group inclusion required similar criteria, excluding diabetes and infections. All participants were excluded if they had genetic disorders, any infections or inflammatory diseases in the reproductive tract.

Results: The mean age of participants was 33.78 ± 5.29 yr, and the mean body mass index was 24.29 ± 3.24, with no significant differences among the groups ($p \geq 0.05$). Sperm concentration and morphology were significantly higher in the control group compared to the infertile groups ($p < 0.001$). Morphological parameters were particularly poor in the teratozoospermic group relative to the oligoasthenoteratozoospermic group ($p < 0.01$). The asthenoteratozoospermic and oligoasthenoteratozoospermic groups demonstrated significantly higher rates of immotile sperm and lower motility compared to controls ($p < 0.001$). Chromatin assessments revealed significantly elevated proportions of chromomycin $A3^+$ and toluidine $blue^+$ spermatozoa in all infertile groups compared to the control group ($p < 0.001$), indicating compromised chromatin structure. Total antioxidant capacity levels were substantially reduced in the infertile groups, while total antioxidant capacity and total oxidant status concentrations were significantly elevated ($p < 0.05$). The oxidative stress index remained balanced in the control group (0.78 ± 0.09), but it was markedly higher in the experimental groups (2.77 ± 0.41; $p < 0.001$). Negative correlations were observed between key semen parameters (concentration, motility, morphology) and chromatin damage (chromomycin $A3^+$, toluidine $blue^+$) as well as total antioxidant capacity and total oxidant status levels. In contrast, total antioxidant capacity showed a strong positive association with these semen quality indicators.
Conclusion: The findings suggest that even a single abnormal semen parameter, such as teratozoospermia, can significantly disrupt oxidative balance and damage DNA/chromatin structure in sperm cells. Monitoring oxidative stress biomarkers alongside DNA integrity assessments could offer valuable insights into male infertility, especially in patients presenting with abnormal morphology.
Keywords: *Chromatin, DNA fragmentation, Male infertility, Oxidative stress, Spermatozoa.*

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O-22
Evaluating chromosomal anomalies and variants in Iranian couples with history of recurrent pregnancy loss

Iravani F, Namjoo Kh, Ghasemi N, Montazeri F, Kalantar SM.
1.*Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
Email: smkyzd@gmail.com

Background: Chromosomal polymorphisms refer to variations in the chromosomal heterochromatin regions and their higher prevalence in infertile cases suggests they may have implications for reproductive health.
Objective: To determine different types of chromosomal anomalies and variants in couples with a history of recurrent pregnancy loss (RPL).
Materials and Methods: This retrospective study analyzed 7,178 infertile couples referred to the Yazd Reproductive Sciences Institute cytogenetic lab (Yazd, Iran) for comprehensive cytogenetic screening from 2016-2024, and 3,073 cases with a history of RPL were included in the analysis. Cytogenetic maps were assessed using GTG banded metaphase karyotyping on peripheral blood lymphocyte cultures. Chromosomal anomalies and variants, sex distribution and age dependency were compared across three RPL abnormal karyotype groups, including: < 3 miscarriages, between 3-5, and > 5 miscarriages.
Results: The assessment in the RPL group data demonstrated that out of a total of 3,073 RPL individuals, 277 (9.01%) cases displayed aberrations in their cytogenetic profile. This included 44 cases (15.88%) with structural aberrations, 16 cases (5.78%) with numerical abnormalities, 1 case (0.36%) with complex abnormality (≥ 2 aberrations), and 216 cases (77.98%) with heteromorphic variants. Among the RPL couples with chromosomal aberrations, men accounted for 56.32% of aberrations, indicating a skew towards male associations in RPL ($p = 0.591$). Age analysis revealed no significant correlation between maternal age and miscarriage frequency; average ages were 35.92, 35.58, and 32.67 yr across the ($RPL < 3$), ($3 \leq RPL \leq 5$), and ($5 < RPL$) groups ($p = 0.148$).
Conclusion: This study underscores the importance of comprehensive cytogenetic evaluations in RPL management. Additional research is required to elucidate the specific mechanisms through which chromosomal variations affect reproductive success.
Keywords: *Infertility, Recurrent pregnancy loss, Chromosome aberrations, Genetic polymorphism.*

O-23
Destructive effects of superovulation on the quality of oocytes and developmental potential of embryos conceived by different assisted reproductive techniques

Jahanbakhsh Asl E[1], Gaffari Novin M[2].
1.*Department of Yas Infertility Center, Shohada Hospital, Qom University of Medical Sciences, Qom, Iran.*
2.*Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Email: e.jahanbakhsh@gmail.com

Background: Embryonic and fetal development can be highly affected undoubtedly by the assisted reproductive

technologies (ART). A wide range of destructive side effects of ovarian stimulation by gonadotropins as an integral part of most ART programs on reproductive indexes/parameters including oocyte and embryo quality and developmental potential of pre- and post-implantation embryos have been reported so far by recent studies. Exogenous gonadotrophins were identified among the one of environmental factors affecting epigenetic reprogramming and imprinting process in maturing oocytes and can potentially induce phenotypic defects in offspring.

Objective: The first aim of this study was to compare the expression of 2 growth factor genes and 3 genes implicated in methylation modification during early developmentation in oocytes produced by superovulation and natural ovulation. The next aim was to compare the expression patterns of some candidate genes involved in embryonic development and imprinting process among embryos produced by different ART techniques.

Materials and Methods: A comparative study were done with a total of 180 MII oocytes produced by super and spontaneous ovulation obtained from at least 20 female *B6D2F1* (*C57BL/63DBA/2*) strain mice (6-8 wk). 150 blastocyst-stage embryos were produced after in vitro culture of oocytes fertilized by somatic cell nuclear transfer (SCNT), intracytoplasmic sperm injection (ICSI), in vitro fertilization (IVF) and in vitro culture of in vivo fertilization (IVC) from at least 50 female and male *B6D2F1* strain mice (6-8 wk of age). Blastocysts obtained from naturally mated female without gonadotrophin treatment were also used as controls. The mRNA expression patterns of candidate genes were accomplished using real time PCR.

Results: The rates of cleavage to blastocyst stage formation were significantly different ($p < 0.050$) between superovulated and naturally ovulated oocytes in SCNT and ICSI-derived embryos. Significant decreasion (at $p < 0.050$ and $p < 0.010$) and destructive effect of superovulation on expression patterns of candidate genes were detected in 60 ooytes and at least 50 blastocyst stage embryos obtained by different methods.

Conclusion: It was shown that superovulation affected both the evolution potential and Genetic characteristics of ART conceived embryos.

Key words: *ART, Superovulation, Epigenetics.*

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O-24
Investigating the expression level of N-methyl d-aspartate glutamate receptors gene and its correlation with sperm motility in asthenozoospermic men

Abbasi F[1], Momeni HR[1], Etemadi T[1], Mosayebi Gh[2].
1.*Department of Biology, Faculty of Science, Arak University, Arak, Iran.*
2.*Department of Biology, Faculty of Sciences, Arak University of Medical Sciences, Arak, Iran.*
Email: fatemehabbasi642@gmail.com

Background: Asthenozoospermia which is characterized by reduced motility is a condition that affects male fertility. Decreased motility is associated with several factors such as oxidative stress, hormonal imbalance, and genetic disorders. The N-methyl d-aspartate glutamate receptors (*NMDAR*) are ligand-gated cation channels that cause calcium influx in nerve cells. According to immunocytochemistry data, *NMDARs* are present in the tail and midpiece of human sperm. Studies revealed that *NMDAR* agonists increase intracellular calcium and affect sperm motility in newts, and human sperm. Intracellular calcium is one of the crucial factors in the regulation of sperm function, especially sperm motility. Although the actual roles of *NMDAR* in human sperm function are unknown, their involvement in calcium influx can be associated with some parameters in human sperm that need calcium.

Objective: This study was conducted to investigate the expression level of the *NMDARs* gene in asthenozoospermic men and its correlation with sperm motility for the first time.

Materials and Methods: In this experimental study, sperm samples were collected from men referred to the Arak Educational, Cultural and Research Center, Arak, Iran. The subjects were divided into 2 groups: normal and asthenozoospermic. The expression level of the *NMDAR* gene in sperm samples in 30 normal men (control group) and 30 asthenozoospermic men was evaluated using quantitative reverse transcription polymerase chain reaction. In addition, the progressive motility of sperm in both groups was evaluated according to the World Health Organization guidelines and expressed as a percentage.

Results: Our results revealed a decrease in the expression level of *NMDARs* gene in the sperm of asthenozoosperm men compared to the normal group ($p < 0.05$). In addition, Pearson's correlation coefficient showed a positive correlation between the expression of this gene and human sperm motility.

Conclusions: Based on our results, sperm motility is associated with *NMDAR* gene expression in asthenozoospermic men. It can be concluded that *NMDARs* (as calcium channels) may be associated with reduced sperm motility in these men.

Keywords: *Human sperm, Asthenospermia, Sperm motility, NMDA receptors gene.*

O-25
Endometrial stromal cells-derived extracellular vesicles mediate bystander decidual reaction and modulate functional features of natural killer cells in a pregnancy-friendly manner

Mousavi M[1], Vanaki N[1], Zarnani K[2], Shokri F[1], Razavi SA[1], Zarnani AH[1, 3].

1.*Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
2.*School of Biology, College of Sciences, University of Tehran, Tehran, Iran.*
3.*Reproductive Immunology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.*

Email: zarnania@gmail.com

Background: Decidualization, the process of endometrial stromal cell (EnSCs) differentiation by steroidal sex hormones, is crucial for blastocyst-uterine cross-talk, implantation, placental development and endometrial immune modulation. Defective decidualization is associated with reproductive failure. Despite research, the contribution of endometrial-secreted factors to decidualization remains unclear.

Objective: Here, we investigated the role of EnSCs-derived extracellular vesicles (EVs) in promoting decidualization and regulating natural killer cell (NK) functions.

Materials and Methods: In this experimental study, endometrial biopsies were obtained via hysteroscopy from 20 women aged 20-40 yr, in the luteal phase, who were referred to an IVF clinic. The study was conducted at the Immunology Department, Tehran University of Medical Sciences, Tehran, Iran between March 2021 and July 2024. Biopsies were performed to isolate EnSCs for downstream analysis. After confirming inclusion and applying exclusion criteria (including infections, autoimmune disorders, recent chemotherapy, corticosteroid or hormone therapy), EnSCs were isolated and characterized for the expression of mesenchymal (CD10, CD73, CD90), hematopoietic (CD34, CD45), cytoskeletal (cytokeratin/vimentin), proliferation (Ki67) and HLA-G markers using flow cytometry and immunostaining. Decidualization was induced in vitro and validated by measuring prolactin secretion and gene expression of prolactin and insulin-like growth factor binding protein 1. Amino acid metabolomics, glucose uptake and lactate production, and pro-inflamatory cytokine levels were assessed using liquid chromatography-tandem mass spectrometry, calorimetric assay, and enzyme-linked immunosorbent assay. EVs were isolated and characterized by expression of TSG101, CD63, and CD81 (western blot), ultrastructure (scanning electron microscopy), size and zeta-potential (dynamic light scattering). PKH26-labeled EVs uptake by EnSCs and NKs at different time points was monitored by fluorescent tracking. EVs derived from decidualized and undecidualized EnSCs at different intervals were tested alone or combined with decidualization inducers (cyclic adenosine monophosphate, medroxyprogesterone acetate, and 17β-Estradiol) to evaluate their effect on decidualization. NKs were isolated by negative selection and the effects of differentiated and undifferentiated EVs on marker expression, proliferation, and cytotoxicity against EnSCs and K562 cells by IL-15-primed NK cells were analyzed using flow cytometry, carboxyfluorescein succinimidyl ester staining, and Calcein-AM staining.

Results: EnSCs showed mesenchymal and vimentin markers but lacked hematopoietic and cytokeratin markers. Decidualization reduced EnSCs proliferation and induced *HLA-G* expression after *IFN-γ* pre-treatment, altered amino acid metabolism (notably increased phenylalanine), and decreased glucose uptake and lactate production. EVs (average size 89.17 nm, zeta potential ~38.15 mV) were more abundantly produced by decidualized cells compared to undecidualized cells. EVs uptake peaked at 4 hr in EnSCs and 24 hr in NK cells. Decidualized EVs, unlike those from undifferentiated cells, enhanced decidualization ($p < 0.0001$). We found a negative correlation between the decidualization capacity of EnSCs and the production of IL-6 and IL-8. EVs derived from EnSC exerted no significant effect on NK cell proliferation or expression of CD16, CD107a, Perforin, Granzym A/B and IFN-γ. More importantly, however, they reduced the cytotoxic effect of NK cells on K562 and EnSCs cells ($p = 0.015$) and increased the frequency of CD56 bright NK cells ($p = 0.007$).

Conclusion: This study reveals unprecedented evidence that EnSCs-derived EVs mediate bystander decidualization and modulate NK cell functions in a pregnancy-friendly manner.

Keywords: *Endometrium, Decidualization, Metabolomics, Extracellular vesicles, Natural killer cells.*

O-26
Exploring the expression profile of *hsa_circ_0004712* as a biomarker for recurrent implantation failure in women who underwent in vitro fertilization

Rezaee S, Babaei E.

Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

Email: babaei2539@gmail.com

Background: Recurrent implantation failure (RIF) is a significant challenge in assisted reproductive technologies, characterized by the inability to achieve successful implantation after multiple transfers of high-quality embryos during in vitro fertilization (IVF) cycles. IVF treatments are costly and often require multiple attempts to result in pregnancy, leading to financial strain, emotional distress, and psychological hardship for many couples. Despite ongoing research, the exact causes of RIF remain poorly understood, complicating the optimization of IVF outcomes. Emerging research suggests that non-coding RNAs (ncRNAs), especially circular RNAs (circRNAs), play

vital roles in regulating implantation processes. CircRNAs are a subclass of ncRNAs with a unique covalently closed-loop structure, which provides stability and resistance to exonuclease degradation, making them ideal candidates for non-invasive biomarkers in biological samples such as blood, urine, and saliva.

Objective: This study aimed to investigate the expression profile of *circ_0004712* in the peripheral blood of women with RIF, comparing it to women who achieved a successful pregnancy following their first IVF attempt.

Material and Methods: A total of 60 peripheral blood samples were collected from women undergoing IVF at Tabriz Valiasr hospital, Tabriz, Iran between June 20, 2024, and March 20, 2025. The study population consisted of 2 groups: 30 women with RIF and 30 women with successful implantation outcomes as controls. Real-time polymerase chain reaction was used to measure *circ_0004712* expression levels in blood samples from both groups. Additionally, bioinformatics analysis was conducted to predict miRNAs targeted by validated circRNAs and to investigate circRNA-miRNA-mRNA interactions. The expression of *PDE7B*, the host gene of *circ_0004712*, was also analyzed to examine significant differences between the two groups.

Results: The quatitative real-time polymerase chain reaction analysis revealed a significant difference in the expression levels of *circ_0004712* between the RIF and control groups. Bioinformatics analysis also demonstrated significant differences in the expression of *PDE7B*, the host gene of *circ_0004712*, between the RIF and control groups.

Conclusion: These results suggest that the dysregulated expression of *circ_0004712* and its host gene *PDE7B* may play a role in the molecular pathways underlying implantation failure.

Keywords**:** *Recurrent implantation failure, Circular RNAs, circ_0004712, In vitro fertilization.*

O-27
Fabrication and characterization of polymer-based nano particles for gene delivery to zygote

Torabi A[1], Bagherzadeh MA[2], Golestan Jahromi M[3], Alipour M[1, 3].

1.*Department of Biochemistry, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.*
2.*School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.*
3.*Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran.*

Email: m.alipour@jums.ac.ir; Mohsen.alipoor2013@gmail.com

Background: Today, gene delivery to animal zygotes for research and therapeutic purposes is of great importance, as researchers strive to engineer various genes in embryos to produce transgenic animal models of human diseases. However, one of the chalenges of gene transfer is the presence of multiple barriers, both inside and outside the cells. To date, various approaches, such as microinjection and the use of nanocarriers, have been employed to overcome these barriers. Among these approaches, and nanocarriers have demonstrated high efficiency in facilitating gene transfer to cell.

Objective: In this research, we aim to fabricate and characterize polymer-DNA nano-complexes to facilitate the gene transfer process to mouse zygotes.

Material and Methods: Initially, we isolated a gene reporter-harboring plasmid white concenteration of 200 ng and high purity. In the next step, DNA was complexed with a polymer to fabricate nanoparticles through a self-assembly process. Subsequently, the formation of these nanoparticles was evaluated using agarose gel electrophoresis, dynamic light scattering, and transmission electron microscopy analyses.

Results: The results from gel electrophoresis demonstrated the successful binding of DNA to the nancarriers. More importantly, dynamic light scattering and transmission electron microscopy confirmed the formation of nano particles with a size of approximately 200 nm and a positive charge. The physicochemical characteristics of the nanocarrier are well-suited for gene delivery to zygotes, which will be prepared using in vitro fertilization methods.

Conclusion: It appears that the results of this study could represent an effective step in facilitationg the production processes for animal models of human diseases.

Keywords: *Gene delivery, Nanocarrier, Animal model, Zygote.*

O-28
Distinguishing features of endometrial and menstrual blood stromal cells identified through decidualization profiling

Vanaki N[1], Tavangar B[2], Darzi M[2], Arefi S[3], Mirzadegan E[2], Mousavi M[1], Shokri F[1], Zarnani AH[1, 4].

1.*Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
2.*Nanobiotechnology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.*
3.*Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
4.*Reproductive Immunology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.*

Email: zarnania@gmail.com; zarnania@sina.tums.ac.ir

Background: Decidualization, the process of endometrial stromal cell (EnSCs) differentiation, is critical for embryo-endometrium cross-talk and fetal development, as well as the functionality of EnSCs.

Although considered as a surrogate for and share many biological features with EnSCs, menstrual blood stromal cells (MenSCs) are a distinct population.

Objective: Here, we investigated the potential link between EnSC and MenSC cell types in terms of decidualization capacity.

Materials and Methods: An observational study was conducted to compare stromal cells isolated from human endometrial biopsies (n = 10) and menstrual blood (n = 20). Both cell types were characterized for mesenchymal stem cell markers and then subjected to *in vitro* decidualization using 2 famous protocols for 6 and 12 days. EnSC and MenSC sources were classified into 2 categories, well-decidualized (WD) and poor-decidualized (PD), based on their capacity to secrete prolactin, as a known and valid decidualization marker. The extent of decidualization as well as the impact of decidualization on senescence, inflammation, metabolome, and glycolysis intermediates, were compared before and after exposure to selected concentration of six senomorphics in all sources as well as WD and PD EnSCs and MenSCs.

Results: Our data indicated that MenSCs have distinct decidualization kinetics compared to EnSCs with faster, limited and sustained capacity to decidualize for an extended period. By day 6 of decidualization, EnSCs exhibited metabolic changes comparable to those of MenSCs by day 3. Decidualization triggered significant differential alterations in glycolysis-associated metabolites, senescence, and interleukin-6 production in EnSCs and MenSCs especially in PD sources. This was also the case for EnSCs and MenSCs in general and PD and WD cells when they were treated with senomorphic agents. The timing of exposure to senomorphics significantly impacted decidualization outcomes, with treatment during decidualization exerted a negative impact on cell senescence. Of note, pretreatment with senomorphics increased senescence in WD EnSCs, while it exerted an opposite effect in PD EnSCs. Senomorphics shifted metabolome of PD EnSCs toward WD EnSCs.

Conclusion: This is the first report notifying that EnSCs and MenSCs differ significantly in their decidualization potential, metabolome, senescence, and responses to senomorphics and provides clinical clues for using senomorphics in women with impaired decidualization and reproductive failure.

Keywords: *Decidualization, Stromal cells, Endometrium, Menstrual blood, Senomorphics, Senescence.*

O-29
Immunomodulatory properties of amniotic fluid-derived mesenchymal stem cells over the lifespan

Zare E[1, 2], Azad FS[2, 3], Montazeri F[2], Hoseini SM[4].

1.*Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
2.*Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
3.*Department of Medical Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
4.*Biotechnology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Email: sm.hoseini@gmail.com

Background: Senescence, a biological process involving the irreversible arrest of cell division, significantly influences the properties of amniotic fluid-derived mesenchymal stem cells (AF-MSCs). These cells are widely studied in regenerative medicine due to their multipotent differentiation capacity and immunomodulatory abilities. However, as AF-MSCs undergo senescence, their functional efficiency declines, affecting not only their proliferation and differentiation but also their immune-regulating functions. The gene forkhead box M1 (*FoxM1*), known for its role in cell cycle progression, is implicated in the regulation of senescence. This study investigates the impact of *FoxM1* on the expression of key immunomodulatory genes, including interleukin-6 (*IL-6*), Cyclooxygenase-1 (*COX1*), and human leukocyte antigen-G (*HLA-G*), during the senescence of AF-MSCs.

Objective: This research aimed to compare the expression of certain genes involved in immunomodulatory and cell senescence between proliferative and senescent AF-MSCs.

Materials and Methods: In this in vitro experimental study, AF-MSCs were obtained from 7 pregnant women by amniocentesis from Yazd Reproductive Sciences Institute, Yazd, Iran. The homogeneity of AF-MSCs was confirmed by flowcytometry. After consecutively passages, the senescence status of AF-MSCs was evaluated by beta-galactosidase (X-gal Cinna Gene) staining. quantitative polymerase chain reaction technique was used to investigate the changes in the expression of senescence and immunomodulatory marker genes.

Results: The molecular analyses revealed a significant upregulation of immunomodulatory genes *IL-6* ($p = 0.008$) and *COX1* ($p = 0.030$) during senescence. Conversely, the increase in *HLA-G* expression did not reach statistical significance ($p = 0.106$). Additionally, the expression of the *FoxM1* gene, associated with cellular senescence, was notably downregulated in senescent cells compared to their proliferative counterparts.

Conclusion: These findings indicate that, despite the cessation of proliferation due to replicative senescence, AF-MSCs derived from varied fetal progenitor cells may maintain their immunomodulatory properties

during in vitro expansion. This characteristic holds significant implications for the therapeutic application of AF-MSCs in regenerative medicine.

Keywords: *Mesenchymal stem cells, Amniotic fluid, Cellular senescence, Forkhead transcription factors, Immunomodulation.*