

## Poster Presentation

### 11<sup>th</sup> Yazd International Congress and Student Award on Reproductive Medicine

#### P-1

#### Effects of aluminum chloride exposure on the micro-structure of the testis in mice

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**Background:** Aluminum, the third most abundant element in the Earth's crust, is found in kitchen utensils, food additives, pharmaceuticals, water purification systems, and cosmetics like anti-perspirants. Upon entering the stomach, aluminum converts to aluminum chloride (AlCl<sub>3</sub>) by gastric acid. Exposure to aluminum can cause various adverse effects, notably reproductive toxicity.

**Objective:** This study aimed to investigate the effect of AlCl<sub>3</sub> on the structure of seminiferous tubules in mice.

**Materials and Methods:** In this experimental study, 15 male NMRI mice, (3 months, 35 ± 3 g), were randomly assigned into 3 groups (n = 5/each): Control (C), experiment 1 (EXP1), and experiment 2 (EXP2). Mice were housed under standard condition at 22 ± 2°C with a 12-hr light/dark cycle and free access to food and water. Mice were treated for 3 wk with daily doses of 0, 50, and 100 mg/kg body weight of AlCl<sub>3</sub> in distilled water (0.1 ml) via intraperitoneal injection. Changes in body weight were recorded. At the end of the treatment period, following CO<sub>2</sub> exposure mice were euthanized via cervical dislocation, and testicular tissue samples were collected. After fixation in 10% buffered formalin and tissue processing, 5 µm sections were prepared and stained with hematoxylin and eosin method. Histological changes in the seminiferous tubules and interstitial tissue were evaluated, and the tubular differentiation index (TDI) was calculated.

**Results:** The control group showed a significant increase in body weight (38.8 gr) during treatment (p < 0.05), while the EXP1 experienced a non-significant decrease (34.96 gr), and the EXP2 group demonstrated a significant reduction (32.19 gr) in body weight (p < 0.05). Micrographs of testicular sections from the control group revealed healthy structure and layers of spermatogenic cells of seminiferous tubules and spermiogenesis occurring near the lumen. The interstitial tissue contained cohesive clusters of Leydig cells. In EXP1, a reduction in seminiferous tubules diameter beneath the tunica albuginea was observed, along with fewer spermatogenic cells, cell layer detachment, shedding of cell layers, and the formation of empty spaces in the tubule epithelium. Mild

interstitial edema accompanied a reduction in Leydig cell numbers. EXP2 exhibited severe tissue damage, with some tubules lacking spermatogenic cells and only Sertoli cells visible among empty spaces. Extensive edema in the interstitial tissue was accompanied by scattered Leydig cells. TDI values among the groups were 95.9 ± 2.9%, 86.1 ± 8.8%, and 51.1 ± 7.3%, respectively, indicating a significant reduction in EXP2 compared to control and EXP1 (p < 0.05).

**Conclusion:** AlCl<sub>3</sub> exposure adversely affects the structural integrity of seminiferous tubules in mice, leading to significant changes in testicular histology. Decrease in TDI emphasizes the potential reproductive toxicity of AlCl<sub>3</sub>, which may compromise male fertility. These findings call for further research into the mechanisms of aluminum-induced reproductive toxicity and underscore the need for regulating aluminum exposure to safeguard male reproductive health.

**Keywords:** Aluminum chloride, Testis, Mice, TDI, Seminiferous tubules.

#### P-2

#### A new approach to Peyronie's disease

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Peyronie's disease is a medical condition characterized by the formation of scar tissue inside the penis, which can lead to a curvature or deformity of the penis during erection. The disease can lead to problems with sexual function and marital relationships, but it does not directly lead to infertility. This narrative review was conducted in the electronic databases Google scholar, PubMed, Web of Science after using the keywords Peyronie's disease, marital relationships and infertility. Initially, 78 articles were identified from 2015-2024 in the fields and articles in English. After the initial review, 34 studies were reviewed. After further exclusion based on abstract review, the full text of 11 published articles that were available was studied. We present seven approaches: 1) intraregional therapies: recent advancements focus on using injectable therapies directly into the plaque causing Peyronie's disease. 2) shockwave therapy: low-intensity shockwave therapy is emerging as a non-invasive option for treating this disease. This technique uses sound waves to stimulate blood flow and promote healing in the affected area. Studies suggest that low-intensity shockwave therapy may reduce pain and improve curvature by promoting tissue remodeling and reducing plaque size over time. 3) vacuum erection devices: can be used not only for erectile dysfunction but also for Peyronie's disease. 4) penile traction therapy: penile traction devices are designed to stretch the penis gently. 5) surgical options: Newer surgical techniques, such as the plication procedure or plaque incision and grafting, aim to correct curvature effectively while preserving penile length. 6) psychological support and counseling: therapy can help

address feelings of anxiety, depression, and body image concerns that often accompany this condition, providing a holistic approach to care. 7) combination therapies: a promising approach involves combining different treatments. These new approaches to treating Peyronie's disease reflect a growing understanding of the condition and the need for individualized care. Incorporating both medical and psychological perspectives can lead to improved outcomes for patients.

**Keywords:** Peyronie's disease, Marital relationships, Infertility.

### P-3

#### The effect of consecutive ejaculation on the sperm parameters in the oligo-astheno-teratozoospermia men

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**Background:** Recently, the World Health Organization recommendation for abstinence time for semen analysis has been challenged in some studies and many recommended a second short abstinence ejaculation. More evidence is needed to approve this for clinical use.

**Objective:** This study aimed to compare the average routine abstinence time (2-7 days) with the short time (1-2 hr) on sperm quality based on functional parameters in a population of oligo-astheno-teratozoospermia (OAT) men.

**Materials and Methods:** From April to July 2024, the semen samples were retrieved from 50 men with OAT 2 times: one standard 2-7 days (long ejaculation) and short duration trimming (1-2 hr after the 1<sup>st</sup> ejaculation). All semen parameters and sperm DNA integrity were compared between groups.

**Results:** Mean sperm concentration (10.40 vs. 8.76,  $p = 0.001$ ), total sperm count (28.53 vs. 12.24,  $p < 0.001$ ), and mean semen volume (2.69 vs. 1.40,  $p < 0.001$ ) were higher in the first ejaculation (2-7 days of abstinence), while progressive motility (20.52 vs. 13.32,  $p < 0.001$ ), non-progressive motility (53.46 vs. 48.86,  $p < 0.012$ ), morphology (2.46 vs. 1.46,  $p < 0.001$ ), and viability (83.90 vs. 77.96,  $p < 0.001$ ) were significantly higher in

the second ejaculation. The second sample also showed lower immotile (26.82 vs. 38.02,  $p < 0.001$ ) and DNA fragmentation (19.5 vs. 26.96,  $p < 0.001$ ).

**Conclusion:** Taking all data into account, an additional short abstinence period may be a simple and helpful strategy to obtain better sperm quality in male infertility causes, especially in OAT cases. The recommended current guidelines regarding the abstinence period may need to be revisited in severe male factors.

**Keywords:** Semen analysis, Consecutive ejaculates, Sexual abstinence, Asthenozoospermia, DNA fragmentation.

### P-4

#### Efficacy of zeta potential sperm selection method on sperm DNA fragmentation

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**Background:** The sperm DNA fragmentation index (DFI) is a male reproductive health criterion affecting assisted reproductive techniques' results. Protamines are proteins found in the nucleus that are crucial for maintaining the integrity of sperm DNA; they are essential for the stability and packaging of sperm DNA. Defects in chromatin compaction are often associated with compromised protamine levels. These defects can lead to damaged DNA strands. Efforts to obtain high-quality mature sperm seem necessary. In this regard, advanced sperm selection techniques, such as zeta potential, have gained popularity.

**Objective:** This study aimed to investigate the effect of zeta potential sperm selection in obtaining spermatozoa with better DNA integrity and chromatin structure.

**Materials and Methods:** This before-and-after laboratory study was performed on 20 infertile men with a high DFI ( $> 25\%$ ) and at least one prior failed in vitro fertilization attempt. Semen samples were processed using the zeta potential technique. Sperm DNA integrity was evaluated pre- and post-treatment using the sperm chromatin dispersion test, and protamine deficiency was assessed using chromomycin A3 staining. The study was conducted at Royan Research Institute, Tehran, Iran from August 2024 to December 2024.

**Results:** Sperm chromatin dispersion results showed that the difference in the mean percentage of total DFI before sperm preparation using the zeta method was  $41.46\% \pm 8.60\%$  and after it was  $32.07\% \pm 11.12\%$  ( $p < 0.001$ ). The average rate of chromomycin A3+ before and after zeta were  $40.31\% \pm 10.87\%$  and  $30.93\% \pm 11.86\%$ , respectively ( $p < 0.001$ ). Statistical significance was defined as ( $p < 0.05$ ).

**Conclusion:** The zeta potential method effectively selected spermatozoa with reduced DNA fragmentation index and with higher quality chromatin.

**Keywords:** Semen, Infertility, DNA fragmentation, Spermatozoa.

## P-5

### Application of nitrates and nitric oxide donors as a transdermal therapy for erectile dysfunction: A comprehensive narrative review

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Erectile dysfunction is recognized as one of the most widespread urological conditions globally, exerting a substantial impact on the quality of life for affected individuals and their partners. With a growing number of men experiencing erectile dysfunction, various pharmacological treatments have emerged in recent years. Nevertheless, systemic therapies continue to pose significant issues, including potential drug interactions and contraindications in patients with comorbid conditions. These challenges have prompted increasing interest in developing formulations that maintain therapeutic efficacy while minimizing systemic side effects and risks. This comprehensive narrative review explores the transdermal application of nitrates and nitric oxide donors for treating erectile dysfunction, focusing on therapeutic potential, mechanisms of action, local side effects, and formulation strategies. A targeted literature search was conducted using the PubMed and Scopus databases. The search was limited to English-language studies without a specific time frame and focused on titles and abstracts, excluding MeSH terms to broaden the scope. Search terms included combinations such as "nitrates", "nitroglycerin", "glyceryl trinitrate", and "NO donor" with "topical", "transdermal", "gel", "cream", "ointment", and "erectile dysfunction". Studies were screened based on their scientific quality and thematic relevance, and those addressing transdermal approaches in erectile dysfunction treatment were selected for full review. The analysis highlighted the potential of various organic nitrates, including nitroglycerin and isosorbide dinitrate, as well as nitric oxide donors such as volatile alkyl nitrites, sodium nitroprusside, L-arginine, and linsidomine. The review evaluated their performance

both as standalone agents and in combination therapies. Key areas of analysis included pharmacodynamics, therapeutic efficacy, tolerability, and potential contraindications. Based on current evidence, enhanced transdermal formulations could offer a promising alternative or adjunctive strategy for managing ED. Nevertheless, most existing evidence stems from preclinical studies, highlighting the necessity for future clinical trials to more accurately assess the practical efficacy and safety of these therapeutic strategies.

**Keywords:** Erectile dysfunction, NO donors, Nitrates, Nitric oxide, Topical therapy.

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## P-6

### Comparing the reliability of two laboratory methods for assessing sperm count; standard neubauer slide and disposable glass slide

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**Background:** Accurate sperm count assessment is critical in reproductive biology for evaluating male fertility. The neubauer hemocytometer is a standardized tool recommended by the World Health Organization for sperm counting due to its precision and reproducibility. In contrast, the disposable glass slide is often used as an alternative method due to its simplicity and cost-effectiveness; however, it is more reliant on the observer's experience.

**Objective:** This study aimed to compare sperm counts obtained using the standard neubauer slide and disposable glass slide to evaluate the reliability and accuracy of these methods.

**Materials and Methods:** This experimental pilot study examined 30 normal semen samples from men at the Andrology Department of the Yazd Reproductive Sciences Institute, Yazd, Iran in winter 2024, which were collected and analyzed in a controlled laboratory setting. Sperm counting was performed independently by 2 observers using both the standard neubauer slide and the disposable glass slide. The neubauer method analysis was conducted using a grid-based system after immobilizing spermatozoa. In the disposable glass slide method, sperm counts were performed using manual observation under light microscopy without grid assistance. Data were collected and compared between observers and across methods to determine consistency, accuracy, and potential discrepancies.

**Results:** The results showed there was no significant difference in sperm counts between using the standard neubauer and disposable glass slide ( $p = 0.452$ ). However, the sperm count using a disposable glass slide by different investigators will yield different results ( $p = 0.001$ ), while counting using the neubauer slide by different people will yield the same results.

**Conclusion:** Although the results obtained from the 2 methods in this research center were not significantly different, the standard neubauer slide, as endorsed by the World Health Organization guidelines, is recommended as the preferred method for sperm count assessment in clinical and research settings. Standardization through the neubauer chamber ensures more accurate and reliable data, which is essential for an accurate assessment of semen parameters and monitoring treatment outcomes.

**Keywords:** Sperm count, Disposable glass slide.

## P-7

### The relationship between the increase in sperm DNA fragmentation index with different methods of sperm cryopreservation

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**Background:** Both the diagnosis and treatment of male infertility depend on the integrity of DNA. Infertility greatly depends on the preservation of human sperm by cryopreservation. When a male partner exhibits significant abnormalities in semen parameters, human sperm cryopreservation is a crucial component of the therapy of male infertility. Semen and spermatozoa can be cryopreserved in liquid nitrogen using a variety of methods and techniques. Sperm motility will be significantly reduced by the cryopreservation procedures, but other factors such as DNA fragmentation, reactive oxygen species, and membrane and acrosome integrity are also impacted. Compared to fertile men, infertile men's sperm DNA is more susceptible to freezing damage.

**Objective:** This study aimed to compare different methods of sperm freezing on the preservation of DNA integrity in sperm samples from individuals.

**Materials and Methods:** The present blinded experimental study was performed on October 2024 in Royan institute, Tehran, Iran. Sperm samples from 10 human male were collected. We divided the samples into 2 different sperm-freezing protocols group. In the first group, the samples were frozen and stored in liquid nitrogen at  $-196^{\circ}\text{C}$ . In the second group, the samples

were stored in a freezer at  $-80^{\circ}\text{C}$ . In the second group had 2 subgroups: in the first subgroup snap-freeze was performed and stored in the  $-80^{\circ}\text{C}$  freezer and for the second subgroup, samples were placed directly into the freezer. The sperm DNA quality was assessed by the sperm chromatin dispersion.

**Results:** The results of the sperm chromatin dispersion showed that there was a difference in the total percentage of DNA fragmentation index between the different methods of sperm cryopreservation ( $p < 0.05$ ). In the first method, the average percentage of DNA fragmentation index was  $(35.70 \pm 0.08)$ , in the second group; in the first subgroup, the mean DFI was  $(45.90 \pm 0.15)$  and in the second subgroup it was  $(51 \pm 0.14)$ . There was a significant difference between the 2 methods (liquid nitrogen at  $-196^{\circ}\text{C}$  and freezer at  $-80^{\circ}\text{C}$ ) ( $p = 0.001$ ), but the difference between the 2 subgroups of the second method was not significant ( $p = 0.166$ ).

**Conclusion:** Freezing and storing the sperm samples in liquid nitrogen is more suitable for preserving DNA integrity and can create better conditions for sample storage. This method can prevent the formation of ice crystals and cause less damage to the sperm nucleus.

**Keywords:** Cryopreservation, Infertility, DNA fragmentation, Spermatozoa.

## P-8

### Rutin mitigates the adverse effects of the methotrexate chemotherapy on sperm parameters in NMRI mice model

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**Background:** Methotrexate, used to treat cancer and autoimmune disorders, can damage testicular tissue, disrupt spermatogenesis, and cause infertility by reducing cellular antioxidant capacity and increasing reactive oxygen species. Rutin, as a flavonoid, has high antioxidant capacity and reduces the effects of reactive oxygen species on sperm quality.

**Objective:** This study investigated the potential protective effects of rutin, a natural flavonoid with antioxidant properties, on sperm parameters in NMRI mice exposed to methotrexate.

**Materials and Methods:** In this experimental study, 40 adult NMRI male mice (average weight: 31 gr) were randomly divided into four groups ( $n = 8$ ): group 1 (control), groups 2 and 3 received at the end of the 3<sup>rd</sup> wk, a single dose of 20 mg/kg/bw of methotrexate by intraperitoneal injection. Groups 3 and 4 received 100 mg/kg/bw rutin for 35 days by oral gavage every other day. Following dissection, the caudal epididymis was placed in a plate containing Ham's F10 culture medium, and the extracted sperm were analyzed for various sperm parameters.

**Results:** The methotrexate group showed a significant reduction in the mean number, viability, motility, and

membrane integrity of the sperm cells when compared to the control group ( $p \leq 0.001$ ). However, these parameters significantly increased in the methotrexate + rutin group compared to the methotrexate group ( $p \leq 0.000$ ).

**Conclusion:** Our results revealed that rutin, a potent antioxidant, can mitigate the adverse effects of methotrexate on sperm parameters.

**Keywords:** *Spermatozoa, Methotrexate, Rutin, Mice.*

## P-9

### The effects of zinc oxide nanoparticles on taxol induced testicular toxicity in mice

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**Background:** Taxol, with the brand name of paclitaxel, is a chemotherapeutic agent capable of producing reactive oxygen species and disrupting male fertility.

**Objective:** In this study, the role of zinc oxide nanoparticles against the destructive effects of taxol on testicular tissue in adult male NMRI mice was investigated.

**Materials and Methods:** 24 adult male NMRI mice ( $35 \pm 2$  gr, 8 wk) were divided into 4 groups ( $n = 6$ /each): control, taxol (5 mg/kg), zinc nanoparticles (5 mg/kg) and taxol + zinc nanoparticles and treated for 35 days intraperitoneally. At the end of treatment, after anesthetizing the mice, body and testis weight and tissue parameters with stereological technique and testosterone level were evaluated.

**Results:** A significant decrease in the weight and volume of the testis respectively ( $p < 0.05$ ) and ( $p < 0.001$ ), the volume, diameter and height of the germinal epithelium of the seminiferous tubules ( $p < 0.001$ ) and the level of testosterone hormone ( $p < 0.001$ ) and a significant increase in the volume of the interstitial tissue were observed in taxol group compared to the control group ( $p < 0.001$ ). The number of germ cells and spermatogenesis indices in the group treated with taxol showed a significant decrease compared to the control group ( $p < 0.01$ ). The simultaneous treatment of zinc oxide nanoparticles with taxol significantly reduced the mentioned parameters compared to the taxol group.

**Conclusion:** These findings suggested an antioxidant potential role for zinc oxide nanoparticles in the protection of taxol -induced testicular toxicities.

**Keywords:** *Taxol, Zinc oxide nanoparticles, Testis, Spermatogenesis indices, Mice.*

## P-10

### The effects of theobromine on sperm biochemical factors in asthenozoospermic men during cryopreservation

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**Background:** Asthenozoospermia reduces sperm motility, leading to infertility. Sperm cryopreservation is an important technique in assisted reproductive technology which often causes significant damage to sperm quality. Adding antioxidants to the cryopreservation medium may mitigate these adverse effects. On the other hand, Theobromine (TB) is a plant alkaloid from the methylxanthine group, and is widely used for its antioxidant properties.

**Objective:** This Experimental study aimed to assess the effect of TB supplementation on sperm biochemical parameters in asthenozoospermia men during cryopreservation.

**Materials and Methods:** Semen samples were collected from 30 asthenospermic men in 1403 at Mehr Infertility Treatment Center, Rasht, Iran. Each sample was then divided into three groups ( $n_1, n_2, n_3$ ):  $n_1$ : control (fresh),  $n_2$ : freeze (treated with cryoprotectant), and  $n_3$ : freeze + TB (treated with cryoprotectant and 10 mmol/L TB). 30 samples were examined in each group. In each sample, the level of sperm tumor necrosis factor alpha (TNF- $\alpha$ ), malondialdehyde (MDA) and sperm antioxidant enzymes, including catalase, glutathione, and superoxide dismutase were analyzed using enzyme-linked immunosorbent.

**Results:** The freeze group showed a significant reduction in the mean levels of sperm antioxidant enzymes, along with a significant increase in mean sperm TNF- $\alpha$  and MDA levels compared to the control group ( $p < 0.001$ ). In contrast, the freeze + TB group showed a significant increase in the mean levels of sperm antioxidant enzymes and a significant decrease in mean sperm TNF- $\alpha$  and MDA levels compared to the freeze group ( $p < 0.001$ ).

**Conclusion:** This study found that cryopreservation harms sperm quality by lowering antioxidant enzyme levels and increasing TNF- $\alpha$  and MDA. TB supplementation improved antioxidant levels and reduced TNF- $\alpha$  and MDA, suggesting it may enhance sperm quality in asthenozoospermic men, potentially improving assisted reproductive outcomes.

**Keywords:** *Asthenozoospermia, Spermatozoa, Cryopreservation, Oxidative stress, Theobromine.*

## P-11

### Association between serum vitamin D levels and sperm PRM1 and PRM2 transcript content

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**Background:** There is evidence that focuses on the possible processes governing the relationship between serum vitamin D levels and male fertility.

**Objective:** The present study evaluated the association between serum vitamin D levels with Protamine 1 (*PRM1*) and Protamine 2 (*PRM2*) transcripts abundance in ejaculated spermatozoa.

**Materials and Methods:** In this cross sectional study, serum levels of 25-hydroxy vitamin D and *PRM1* and *PRM2* transcripts abundance were analyzed in ejaculated spermatozoa from normozoospermic donors (n = 45) and infertile men (n = 42) attending the Infertility Research and Treatment Center of ACECR Khuzestan, Ahvaz, Iran from September 2023 to October 2024. Using real-time polymerase chain reaction *PRM1* and *PRM2* transcript content were assessed in ejaculated spermatozoa.

**Results:** In infertile men compared with fertile controls significantly (p = 0.014) lower levels of serum vitamin D concentrations were observed. Serum vitamin D levels were significantly correlated with sperm progressive motility (r = 0.266, p = 0.040) and normal morphology (r = 0.286, p = 0.027). A significant (p = 0.031) lower abundance of sperm *PRM1* transcript was seen in infertile men compared with fertile controls. Men with lower serum vitamin D concentrations showed lower abundance of *PRM1* (p = 0.032) and *PRM2* (p = 0.045) transcripts in ejaculated spermatozoa.

**Conclusion:** Our findings demonstrated the association between serum vitamin D concentrations and *PRM1* and *PRM2* transcripts content in ejaculated spermatozoa. Low levels of serum vitamin D may impact the male fertility by impairment in sperm DNA integrity.

**Keywords:** Vitamin D, Male infertility, DNA integrity, Spermatozoa, Protamine.

## P-12

### Comparative effects of opium tincture and methadone syrup on the reproductive system of male Wistar rats in medication-assisted treatment

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**Background:** Medication-assisted treatment is a comprehensive approach to treating substance use disorders, particularly for opioid dependence. While opium tincture and methadone syrup are frequently prescribed for the treatment of opioid dependence, their effects on the reproductive system remain inadequately understood.

**Objective:** This study aimed to examine the impacts of opium tincture and methadone syrup on plasma testosterone levels and the structural integrity of the testes of rats with addiction.

**Materials and Methods:** In this experimental study, 36 adult male Wistar rats (200-250 gr, 8-10 wk) were

divided into 3 groups (n = 12/each) of a control group, a methadone syrup group, and an opium tincture group. Following the establishment of addiction, rats received the maximum doses of methadone syrup (16.25 mg/kg) and opium tincture (150 mg/kg) administered twice daily for either 4 or 8 wk. After the 4<sup>th</sup> wk, half of the animals from each group were randomly selected for sampling, while the remaining rats were sampled at the end of the 8 wk. Histological examinations of the testes were performed, and serum testosterone levels were measured.

**Results:** Compared to the control groups, serum testosterone levels were significantly decreased in the groups treated with opium tincture after 4 and 8 wk (p = 0.029, 0.034, respectively). However, specific testis weight was significantly reduced in the groups treated with opium tincture after 4 and 8 wk (p = 0.044, 0.040, respective) in comparison to the control groups. Histological assessments indicated that the seminiferous tubule area and the germinal layer area were significantly reduced in rats receiving either opium tincture or methadone syrup during both treatment durations (p < 0.001 for each) also their relative ratios was significantly decreased in rats receiving opium tincture after 4 and 8 wk (p ≤ 0.001, 0.030, respectively) as well as methadone after 4 and 8 wk (p = 0.020, 0.011, respectively) in comparison to control groups. However, the evaluation of spermatogenesis using the Johnson index revealed no significant differences between the studied groups (p > 0.05).

**Conclusion:** The findings of this study indicated that the detrimental effects of oral opium tincture on the reproductive system of male rats were more significant than those associated with methadone syrup.

**Keywords:** Medication-assisted treatment, Opium, Methadone, Addiction medicine, Testis, Rats.

## P-13

### Sperm DNA fragmentation index affects intrauterine insemination outcome: A preliminary data of cohort study

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**Background:** Intrauterine insemination (IUI) is generally the first line of infertility treatment. Sperm

DNA fragmentation index (DFI) have important roles in sperm health and its function such as fertilization capacity. In addition, sperm motility in IUI cases is very important since sperm with better motility has more chance to achieve the oocyte and fertilization.

**Objective:** This study was conducted to investigate the relationship between sperm DFI and sperm motility with IUI outcome.

**Materials and Methods:** In this ongoing cohort study, from 2024-2025, 300 couples whose wives are candidate for IUI at Royan Institute, Tehran, Iran are enrolled and followed up till 2 wk later in day of checking serum beta human chorionic gonadotropin test. Inclusion criteria of the study is < 49 yr old men, not having varicocele, leukocytospermia, or reproductive system infections, with body mass index < 30 kg/m<sup>2</sup>, and < 40 yr old women with body mass index < 30 kg/m<sup>2</sup>. In the day of IUI cycle, non-processed part of semen sample received from embryology lab of Royan Institute, Tehran, Iran and 3-5 ×10<sup>6</sup> sperm were separated for DFI measurement by terminal deoxynucleotidyl transferase dUTP nick end labeling assay. Sperms washed and fixed in paraformaldehyde 4% (1 hr, 4°C), then washed with phosphate buffered saline (PBS) (2500 rpm- 5 min). After smear preparation, permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) was added for 2 min in 4°C and washed with PBS. The slides were incubated in terminal deoxynucleotidyl transferase dUTP nick end labeling reaction mixture (1 hr, 4°C). After washing and mounting slides with glycerol in PBS (1:1), sperms analyzed using fluorescence microscope. According to IUI outcome base on beta HCG test, DFI percentage were compared between IUI success and failure groups. **Results:** Till now, 39 couples were enrolled: 9 couples in the IUI success group and 30 couples in the IUI failure group. The mean of sperm DFI was 5.5 ± 2.3% and 12.13 ± 3.8% in IUI success and IUI failure groups, respectively. This increase in DFI in the IUI failure group was statistically significant (p < 0.0001). There were no significant differences in sperm motility between the 2 studied groups at this sample size (p = 0.93).

**Conclusion:** According to preliminary data, men in the IUI failure group had higher DFI. This study continues until reaching 30 couples in each group.

**Keywords:** IUI, DFI, Sperm motility, TUNEL.

#### P-14

### The influence of sodium hydrogen sulfide on sperm function and oxidative stress in varicocele-induced rats

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**Background:** Infertility is a complex condition with a high prevalence of varicocele which affects approximately 35-40% of cases and refers to the abnormal enlargement of the pampiniform plexus, primarily caused by reversed blood flow in the spermatic veins. While several studies have explored the pathophysiology of varicocele and its association with male infertility at the molecular level, oxidative imbalance and hypoxia seem to be the key factors leading to this condition.

**Objective:** This study aimed to investigate the effects of sodium hydrogen sulfide (NaHS) supplementation, a hydrogen sulfide donor, on oxidant and antioxidant markers, as well as sperm function in rats with experimentally induced varicocele.

**Materials and Methods:** In this experimental study, 55 male Wistar rats were divided into 3 groups: varicocele (25 rats), control (20 rats), and sham (10 rats). In the varicocele group, 5 rats started receiving NaHS treatment immediately after surgery and continued for 4 months. Another 10 rats began NaHS treatment 2 months after surgery and continued until the 4-month mark. The remaining 10 rats in the varicocele group did not receive any treatment. The control and sham groups followed similar treatment schedules. After 4 months, all rats were sacrificed and assessments were made of sperm parameters and function tests, as well as testicular malondialdehyde and total antioxidant capacity.

**Results:** Varicocele induction significantly impaired sperm parameters and sperm function tests. NaHS treatment for 2 months increased sperm concentration (p < 0.001), while treatment for 2 and 4 months improved motility (p = 0.002 and p < 0.001), chromatin status (p = 0.001), and intracellular reactive oxygen species (p < 0.001) compared to untreated varicocele rats. After 4 months, NaHS treatment reduced testicular malondialdehyde levels (p = 0.004). Testicular total antioxidant capacity significantly increased after 2 months (p = 0.025) but decreased after 4 months of treatment in the varicocele group (p = 0.04).

**Conclusion:** NaHS treatment improved sperm parameters and reduced oxidative stress in varicocele rats, with varying effects observed depending on the duration of treatment.

**Keywords:** Varicocele, Semen analysis, Chromatin, Oxidative stress.

#### P-15

### Microsurgical techniques and assisted reproductive methods in male patients with cystic fibrosis: Approaches to fertility restoration

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Mutations in the gene that regulates cystic fibrosis transmembrane conductance result in the hereditary condition known as cystic fibrosis. To improve the condition of individuals with Cystic fibrosis and assist in their fertility, techniques such as microsurgical techniques (including microsurgical sperm retrieval, testicular sperm extraction, and microsurgical varicocelelectomy) and assisted reproductive technologies (such as intracytoplasmic sperm injection, in vitro fertilization, and preimplantation genetic testing) can be used. Moreover, to investigate strategies for improving the quality of life and life expectancy of males living with cystic fibrosis, paying particular attention to their psychological and emotional needs is essential. In addition, in this narrative review study, the most relevant studies (from 692 studies) that were published from 2015-2025 and were accessible through PubMed, Scopus, and Google Scholar were reviewed. Therefore, studies on the use of assisted reproductive technologies and microsurgical procedures have revealed that male fertility in individuals affected by cystic fibrosis is greatly affected. These therapies not only address the physical difficulties related to the disorder but also help to raise general mental health and self-esteem. Additionally, assisted reproductive technologies and microsurgical procedures have greatly raised the chances of conception in male individuals with cystic fibrosis. By means of the meticulous application of sperm retrieval techniques and intracytoplasmic sperm injection, together with extensive genetic counseling, these men can achieve biological parenthood while controlling the related hereditary risks. In conclusion, people faced with cystic fibrosis who had not survived beyond the age of eighteen now have the chance to have fatherhood, therefore representing a major medical breakthrough.

**Keywords:** Cystic fibrosis, Microsurgery, Methods, Assisted reproductive technologies.

## P-16

### Short and long-term effects of COVID-19 and COVID vaccines on male fertility

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The SARS-corona virus-2 enters the testis via angiotensin-converting enzyme 2 receptors. Its destructive mechanisms in the testicles include cytokine storm, systemic inflammation, oxidative stress, and

fever, all of which impair spermatogenesis and disrupt hormonal balance in the short term. Acute coronavirus disease of 2019 (COVID-19) infection leads to testicular inflammation, reduced sperm concentration, and impaired motility. Immune cell infiltration (cluster of differentiation 3 [CD3+], T lymphocytes, cluster of differentiation 68 [CD68+] macrophages, and leukocytes) into the interstitial tissue further damages Leydig cells, reducing testosterone secretion and spermatogenesis. Additionally, fever and local inflammation can cause orchitis, negatively affecting sperm count, motility, and morphology. This study examines the short and long-term effects of COVID-19 on the male reproductive system, including testicular damage, hormonal imbalances, and sperm quality, while also assessing the impact of vaccination on male fertility. The review evaluates the studies on men with acute and chronic COVID-19. Also, the effect of vaccination was assessed and the following reports were evaluated. 1) testicular pathology: electron microscopy detection of viral particles in Sertoli, Leydig, and spermatid cells. 2) sperm analysis: evaluation of leukocytospermia, teratospermia, and obstructive azoospermia. 3) hormonal assessment: disruptions in the hypothalamic-pituitary-gonadal axis and testosterone levels. 4) long-term Effects: investigation of chronic inflammation, fibrosis, and seminiferous tubule damage. 5) vaccine impact: assessment of COVID-19 vaccines on sperm parameters and hormonal balance. The results showed that the short-term effects were: 1) testicular inflammation, leukocytospermia, and reduced sperm quality. 2) viral presence in testicular cells, leading to impaired spermatogenesis. 3) hormonal imbalance due to Leydig cell dysfunction. Also, the long-term effects were 1) potential scar formation, reproductive tract obstruction, and obstructive azoospermia. 2) chronic damage to seminiferous tubules, decreased sperm production, and subclinical fibrosis. On the other hand the studies showed that vaccination had no adverse effects on sperm parameters or hormones reported. COVID-19 significantly impacts male reproductive health in both acute and chronic phases, with potential long-term consequences such as fibrosis and hormonal dysfunction. While vaccines show no harmful effects on fertility, further longitudinal studies are needed to fully understand the lasting implications of COVID-19 and vaccination on the male reproductive system. Timely treatment is crucial to minimizing long-term complications.

**Keywords:** COVID-19, Spermatozoa, Vaccine, Male fertility.

## P-17

### In vitro effect of N-acetylcysteine on the quality and biochemical factors of normal human sperm

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**Background:** Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects a significant portion of the population. Assisted reproductive technologies (ART) offer treatment options in which sperm processing is a critical step. This process usually takes about an hour and involves separating sperm from other components of the seminal fluid. However, exposure to external factors during this step can cause oxidative stress and reduce sperm quality. Therefore, the use of antioxidants is recommended to reduce oxidative stress and increase the success of ART. N-acetylcysteine (NAC), a potent antioxidant containing thiol groups, effectively neutralizes free radicals and protects sperm from oxidative damage.

**Objective:** Given that some normal, fertile men may benefit from ART, this study aims to evaluate the effect of NAC on the quality, biochemical properties, and reactive oxygen species levels of normal human sperm during one hour of in vitro incubation.

**Materials and Methods:** After liquefaction, semen samples from 30 fertile men were divided into 2 groups: control (no treatment, 1-hr incubation) and NAC-treated (50  $\mu$ M NAC, 1-hr incubation). The study assessed sperm parameters, including motility, viability, DNA and chromatin integrity, plasma membrane integrity, and mitochondrial membrane potential. Additionally, total antioxidant capacity, malondialdehyde, and reactive oxygen species levels were evaluated.

**Results:** In the NAC group, compared to the control group, there was a significant increase in the mean percentages of total and progressive sperm motility, chromatin and DNA integrity, cytoplasmic integrity, mitochondrial membrane potential, and total antioxidant capacity levels ( $p < 0.001$ ). Conversely, the mean percentage of non-progressive sperm motility ( $p < 0.001$ ), as well as malondialdehyde ( $p = 0.005$ ) and reactive oxygen species levels ( $p = 0.01$ ), were significantly lower in the NAC group compared to the control group.

**Conclusion:** We concluded that NAC improves sperm quality during in vitro incubation by increasing motility, integrity, mitochondrial function, and antioxidant capacity while reducing oxidative stress markers. These findings suggest NAC is a valuable antioxidant supplement for improving sperm quality and increasing ART success rates.

**Keywords:** N-acetylcysteine, Oxidative stress, Spermatozoa.

## P-18

**The role of probiotics and synbiotics in enhancing semen quality and sperm function in male infertility: Mechanisms and clinical implications**

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**Background:** The prevalence of male infertility has prompted extensive research into therapeutic interventions aimed at improving semen quality. Probiotics, defined as live microorganisms including strains of lactobacillus, bifidobacterium, and lactococcus, and their combinations that confer a health benefit to the host, with prebiotics (synbiotics), have gained attention as a potential therapeutic approach for improving male reproductive health, particularly through their influence on semen parameters through different mechanisms.

**Objective:** This review aimed to analyze clinical and animal studies to examine the effects of probiotics and/or synbiotics on male sperm quality. The results, underlying mechanisms, and future potential of these interventions for male reproductive health were discussed.

**Materials and Methods:** A comprehensive search was conducted across major databases, including PubMed, Google Scholar, and Scopus, using relevant keywords including "male infertility" or "semen" or "sperm" and "probiotic" or "synbiotic" without any restriction on publication date. This search was conducted between January and February 2025. Only original clinical and animal studies published in English were included. Observational, in vitro, and review articles were excluded.

**Results:** Five randomized clinical studies and 5 animal studies met the inclusion criteria. The results have demonstrated significant improvements in sperm quality, especially sperm motility, morphology, and overall count, and also reduction in sperm DNA fragmentation with the use of synbiotics and multi-strain probiotics in males with idiopathic infertility through alleviating oxidative stress, modulating inflammation, regulating reproductive hormones, and restoring gut microbiota balance.

**Conclusion:** This review highlights the diverse mechanisms through which probiotics and synbiotics exert beneficial effects on male reproductive health. While the current evidence is promising, variability in probiotic strains, dosage, duration of treatment, and study designs can make it challenging to draw direct comparisons and generalizations across studies. Further large-scale clinical trials are necessary to fully elucidate the optimal probiotic strains, dosages, treatment durations, and safety of probiotics as a therapeutic approach for male infertility.

**Keywords:** Probiotics, Synbiotics, Semen, Spermatozoa, Male infertility.

## P-19

**Dose-dependent deleterious effects of chronic morphine administration on sperm and testicular parameters in adult male NMRI mice**

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**Background:** Opioid use, particularly morphine, has adversely affected male reproductive health, but its effective destructive dose has not yet been determined.

**Objective:** This study aimed to evaluate the effects of various doses of morphine injections over 45 days on sperm and testicular parameters in mice, providing insights into the reproductive consequences of chronic morphine exposure.

**Materials and Methods:** In this experimental study, 24 adult male NMRI mice, (8-10 wk, 25-30 gr) were randomly assigned to 4 groups (n = 6/each), including vehicle and morphine groups. The morphine groups received morphine at various doses of 10 (M10), 30 (M30), and 40 mg/kg (M40), twice daily (every 12 hr) for 45 consecutive days. Mice in the vehicle group received normal saline under the same schedule. At the end of the treatment period, all animals were euthanized, and sperm samples were collected from the tail of the epididymis for evaluating the sperm parameters (count, motility) using standard sperm analysis techniques. Also, their left testes were isolated, fixed, deparaffinized, sectioned, and stained with hematoxylin/eosin to assess the morphometries of the seminiferous tubules using Image J software.

**Results:** Compared to the vehicle group, a dose-dependent decrease in sperm count was observed in the animals receiving morphine, with the most pronounced reduction in the group receiving 40 mg/kg of morphine (M10; p = 0.0098, M30; p = 0.0005, M40; p = 0.0002). Also, sperm motility decreased significantly in the morphine-treated mice that received 2 high doses of morphine (M30 and M40; p < 0.0001), although no statistically significant difference was found between the vehicle and morphine-10 groups. In addition, a considerable increase in the lumen's diameter (M30 and M40; p < 0.0001) and a marked decrease in the germinal epithelium thickness of seminiferous tubules were observed in the morphine-treated groups (M30; p = 0.0069, and M40; p < 0.0001) compared with the vehicle group.

**Conclusion:** The findings of this study demonstrate that chronic morphine exposure significantly impairs sperm parameters and also testicular morphometry in a dose-dependent manner, particularly at higher doses. These results underscore the potential reproductive risks associated with opioid use, highlighting the need for further investigation into the long-term effects of opioids on male fertility.

**Keywords:** Morphine, Sperm parameter, Reproductive toxicity, Mice.

## P-20

### Investigating the importance of assisted reproductive technology nurses' cultural

### competence in the quality of care for infertile clients: A systematic review

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**Background:** With the rise in global migration and cultural diversity, assisted reproductive technology (ART) nurses must be equipped to understand and address the unique beliefs, values, and practices of their patients. Cultural competence among nurses in ART is increasingly recognized as a critical factor influencing the quality of care provided to infertile clients.

**Objective:** The aim of this study was to investigate the importance of nurses' cultural competence in the quality of care for infertile clients.

**Materials and Methods:** This systematic review was conducted in accordance with PRISMA guidelines. A comprehensive search was performed in PubMed, CINAHL, Scopus, and Google Scholar for English-language articles published between January 2010 and December 2023, using keywords such as "cultural competence", "ART nursing", and "infertility". Studies were included if they empirically examined the link between cultural competence and nursing care in ART contexts. Non-empirical works and unrelated studies were excluded. The quality of included articles was assessed using the Joanna Briggs Institute (JBI) critical appraisal tools. A PRISMA flow diagram documented the screening and selection process.

**Results:** A total of 1,245 records were initially identified, and after removing duplicates and screening, 68 full-text articles were reviewed. Following eligibility assessment, 18 studies were included in the final analysis. The findings highlight that cultural competence among ART nurses significantly enhances communication, fosters patient trust, and improves adherence to treatment. Patients reported higher satisfaction when their cultural backgrounds were acknowledged. Culturally responsive care was also associated with improved clinical outcomes by creating a respectful and inclusive environment for infertile clients from diverse backgrounds. These results underscore the critical role of cultural competence in ART nursing practice.

**Conclusion:** Targeted training programs and the integration of cultural assessments into routine practice. By prioritizing cultural competence, ART nursing can improve patient experiences and outcomes, ultimately contributing to more effective infertility treatments. The findings underscore the need for ongoing education and awareness initiatives to cultivate a culturally sensitive approach within ART nursing, ensuring that all clients receive high-quality, personalized care tailored to their diverse needs.

**Keywords:** Cultural competence, ART nurse, Infertility, Patient care, Nursing practice.

## P-21

### Study of changes of C-reactive protein in using human platelet lysate after cesarean section in dogs

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**Background:** The reproductive physiology of canines is characterized by an anovulatory cycle, exhibiting monoestrus behavior with a mandatory anestrus phase. Cesarean sections are performed for various indications, including obstructive dystocia and uterine inertia, both primary and secondary. This surgical procedure can effectively resolve many dystocia-related complications and enhance neonatal survival. However, cesarean sections are associated with risks such as hemorrhage, retained placenta, adhesions, uterine scarring, incision complications, and metritis, which can lead to delayed uterine healing, reduced fertility, and decreased litter size. C-reactive protein (CRP) is an acute-phase protein that reflects systemic inflammation in dogs following surgery, trauma, infection, or neoplasia. It is also a valuable diagnostic marker for systemic inflammatory responses in canine patients.

**Objective:** This study aimed to assess changes in CRP following the use of human platelet lysate after cesarean section in dogs.

**Materials and Methods:** In this experimental study, 10 pregnant native dogs (3-5 yr, 22-25 kg), were included. The appropriate time of cesarean surgery was determined following pregnancy diagnosis by ultrasonography and evaluation of the date of parturition. These dogs underwent standard cesarean section; by the extraction of puppies through a 4-5 cm ventral incision on the uterine horns. For 5 dogs in the treatment group, platelet lysate was administered at the incision site within the myometrium following Cesarean section. In 5 dogs of the control group, normal saline was injected at the incision site within the myometrium after cesarean surgery. Blood samples were collected from both groups on day zero (before surgery) and 5 days post-surgery. CRP was measured using a canine solid-phase sandwich the enzyme-linked immunosorbent assay method.

**Results:** The mean CRP concentration on day zero in the control group was 3.21 µg/ml, and in the treatment group was 3.18 µg/ml, which was not significantly different. 5 days after the cesarean section, the mean CRP concentration increased in both groups. In the control group, it was 15.73 µg/ml but in the treatment group, it was 13.48 µg/ml, significantly lower than the control group.

**Conclusion:** Platelet-rich plasma and its derivatives such as human platelet lysate have emerged as a promising non-invasive therapeutic option for various medical conditions. Human platelet lysate is acellular and reduces concerns about immune reactions while providing a concentrated source of growth factors and cytokines. Additionally, human platelet lysate can be frozen for long-term storage. According to the result of the present study, it seems that using human platelet lysate in the incision line can effectively reduce inflammation caused by cesarean surgery.

**Keywords:** Incision, Uterine repair, Acute phase protein, Inflammation response, PRP.

## P-22

### Evaluation of vitamin D serum level and its relationship with anti-Müllerian hormone level in infertile women

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**Background:** The key role of vitamin D in fertility is emphasized by the fact that vitamin D receptors are present in male and female reproductive organs, tissues and cells. Although anti-Müllerian hormone (AMH) is a key ovarian reserve marker, studies on the relationship between vitamin D levels and AMH yield conflicting results.

**Objective:** The present study aimed to investigate the level of serum vitamin D concentration in the population of infertile women referred to Shokoufeh Infertility Treatment Center, Shahrood, Iran and its relationship with the serum level of AMH.

**Materials and Methods:** In this cross-sectional study, the data of serum vitamin D and AMH level of 148 infertile women referred to Shokoufeh Infertility Center, Shahrood, Iran between 2021 and 2022 for infertility treatment were extracted from their medical records. All women with polycystic ovary syndrome, endometriosis or history of ovarian surgery were excluded. Finally, the relationship between serum vitamin D and AMH level were evaluated.

**Results:** Among the studied infertile women, 37.2% (n = 55) had vitamin D deficiency, while 62.8% (n = 63) had normal vitamin D levels. The mean serum concentration of AMH was not significantly different between groups (1.8 ± 1.2 vs. 1.9 ± 1.1 ng/ml, respectively, p = 0.396). No significant relationship was observed between serum vitamin D and AMH levels (p = 0.120).

**Conclusion:** Numerous studies have yielded inconsistent results, and the findings of the present study are consistent with those reporting no significant association between vitamin D levels and AMH. While vitamin D may have some influence on AMH expression, it is probable that other regulatory factors exert a stronger impact.

**Keywords:** Vitamin D, Anti-Müllerian hormone, Correlation, Infertility.

## P-23

### Investigating the influencing factors on the clinical pregnancy rate in the intrauterine insemination cycles

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**Background:** Intrauterine insemination is considered one of the procedures of assisted reproductive techniques that help some infertile or hypo-fertile couples. Many factors affect on the pregnancy success rate in intrauterine insemination cycles.

**Objective:** This study evaluated the influence of clinical and laboratory characteristics of cases on clinical pregnancy rate in intrauterine insemination cycles.

**Materials and Methods:** This is a single-center cross-sectional study. The analysis was performed on 119 cases referred to the Infertility Treatment Center in Sari, Iran, between September 2023 and August 2024. The variables evaluated in this study are: age of women and their spouses, causes and type of infertility, sperm count and motility, sperm preparation method, type of medium used for processing and medicine used for ovarian stimulation during the cycle. The relationship between these factors and the rate of clinical pregnancy rate was investigated.

**Results:** The mean age of women and their spouses was  $28.76 \pm 4.9$  and  $32.82 \pm 5.5$  yr, respectively. The type of infertility in women was 63.9% (76 of 119) primary and 36.1% (43 of 119) secondary. The most common causes of infertility were unexplained (28.6%), polycystic ovary syndrome (27.7%), male factor (10.9%) and combined factors of male and PCOS (8.4%). In 66.4% of cases, the sperm sample was prepared by the swim-up method, and in 33.6% the gradient method was used. The sperm count after processing was  $36 \pm 1.5$  m/ml. Sperm forward motility after processing was  $41.8 \pm 1.4$ %. In the cases whose sperm preparation media was Ham's F10, 73.1% Ham's with HEPES and 26.9% Ham's without HEPES were used. The clinical pregnancy rate was 17.6%. All pregnancies were singletons. The rate of clinical pregnancy was 23.8% in women with polycystic ovarian syndrome, 14.3% in

women with unexplained infertility, 14.3% in women with combined factors (male and polycystic ovarian syndrome), 9.5% in cases with endometrioma and 9.5% in cases with hypothalamic amenorrhea. However, this difference was not significant ( $p > 0.05$ ). There was no significant difference in the type of infertility, the number and motility of sperm after processing, the type of media and the method of sperm preparation in the pregnant and non-pregnant groups. The rate of pregnancy was 18.2% when combination of letrozole, clomiphene, and HMG were used for stimulation rather letrozole or clomiphene alone ( $p = 0.02$ ).

**Conclusion:** The data from the present study showed that ovarian stimulation with combined use of letrozole, clomiphene and HMG has positive effect on the clinical pregnancy rate in intra insemination cycles.

**Keywords:** Intrauterine, Insemination, Infertility, Pregnancy.

## P-24

### The impact of the COVID-19 pandemic on healthy reproductive and childbearing services at Shiraz University of Medical Sciences

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**Background:** Providing care services for healthy fertility and offering counseling and care services for infertile women to monitor the treatment process has always been a key activity of the health care system. The outbreak of the coronavirus pandemic and subsequent reduction in activities at the national level posed challenges to the provision of health care services.

**Objective:** This study aimed to evaluate the impact of the coronavirus pandemic on the provision of healthy fertility and childbearing services in the healthcare units of Shiraz University of Medical Sciences, Shiraz, Iran.

**Materials and Methods:** In a cross-sectional study conducted from March 2019 to February 2023, under the auspices of Shiraz University of Medical Sciences, Shiraz, Iran inclusion criteria involved women who have been married for at least 6 months, have no children and are not currently pregnant. Exclusion criteria focused on pregnancy and divorce. Women whose last child is 18 months or older, women who have had a miscarriage in their last pregnancy and women who desire to have children at any age and number of children were included. Services provided to a group of married women aged 10-54 included special care services for healthy fertility, fertility counseling services, identification, counseling, and referral services for infertile individuals, and pre-conception care services. Following fertility counseling, data was collected through the healthy fertility electronic office system and analyzed using statistical methods. A census method was applied to include all documented cases, and variables assessed included key factors such as age, marital status, and medical history. Data analysis was

performed using appropriate statistical methods to ensure a comprehensive evaluation of the recorded data.

**Results:** The data of 420525 women aged 10-54 with spouse who received care were examined. The study revealed an increasing trend in women's referrals for pre-conception care before, during, and after the coronavirus pandemic. However, this increase was not statistically significant during and after the pandemic ( $p = 0.167$ ). Despite a significant increase in the frequency of women eligible for counseling and childbearing before, during, and after the pandemic referrals for counseling showed a significant decrease ( $p = 0.000$ ). The frequency of infertility and the frequency of childlessness did not show a statistically significant difference in the 3 study periods ( $p = 0.264$ ).

**Conclusion:** The results suggest that the Covid-19 pandemic, except for the decrease in women's referrals for counseling services due to fear of contracting Covid-19, had no significant impact on other indicators of special reproductive health care and pre-conception care services.

**Keywords:** COVID-19 pandemic, Reproductive care, Infertility care, Healthcare services.

## P-25

### Unraveling the link between assisted reproductive technology and childhood cancer subtypes

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Assisted reproductive technology (ART) has revolutionized the approach to infertility, but its potential implications for childhood cancer remain a topic of ongoing debate. Previous studies have reported varying associations between ART and several childhood cancers subtypes, highlighting the complexity of this relationship due to confounding factors such as birth weight and sex. The goal of the current study is to present a narrative review on the association between ART and specific childhood cancer subtypes. This review aims to summarize and analyze the existing literature, focusing on the potential genetic and environmental factors that may influence the outcomes, rather than a systematic quantitative analysis. The current narrative review was conducted by searching relevant literature in databases such as PubMed, Scopus, and Google Scholar. The search was guided by keywords related to ART, childhood cancer, specific cancer subtypes, genetic factors, and environmental factors. The impact of ART on childhood cancers is investigated and it has paradoxical results. Some studies prove the impact, specifically the association of the intervention with osteosarcoma without any regard for

birthweight and gestation status. Hepatoblastoma, germ-cell tumors, retinoblastoma and rhabdomyosarcoma are following cancer subtypes but can be confounded strongly by confounding factors like birthweight (hepatoblastoma with low birthweight and neuroblastoma and germ-cell tumours by high birthweight). Also, sex is another confounding factor as it is observed that there is a higher cancer risk in boy than girls in intra cytoplasmic sperm injection technique. Also, genetic disorders are considered to be the main cause of some cancers. Fertility drugs that used for any step of this process can make epigenetic changes specially in imprinted genes that lead to retinoblastoma, neuroblastoma and acute myeloid leukaemia. Some studies didn't find any association between childhood cancers and ART specifically in in vitro fertilization techniques. Leukemia, as the most common childhood cancer, has not any risk increase in association with ART. Clinical evidences suggest an association between ART and childhood cancer subtypes specifically genetically-related subtypes. The most risk belongs to osteosarcoma followed by hepatoblastoma. However, some observations ensure the independence between ART and childhood cancers. The most important concerns in results are related to confounding biases.

**Keywords:** Assisted reproductive technology, Childhood cancer, Pediatric cancer.

## P-26

### Psychometric evaluation of the endometriosis impact questionnaire in an Iranian population

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**Background:** Endometriosis is a benign and chronic gynecological estrogen-dependent disease. Considering the prevalence and the importance of measuring the long-term effects of endometriosis in affected women's lives the endometriosis impact questionnaire (EIQ) scale was designed and psychometrically analyzed in English in Australia, in 3 recall periods (last 12 months, 1-5 yr ago and > 5 yr ago). It has never been used in Iran and its validity and reliability have not been assessed either.

**Objective:** The present study aimed to translate and investigate the psychometric properties of the EIQ.

**Materials and Methods:** In this cross-sectional study, 200 women were selected through random sampling in 2022 in Mashhad-Iran. After forward and backward translation, the face validity, content validity, and construct validity of EIQ (through corrected item-total

correlation) were examined. To assess the reliability of the scale, both internal consistency (Cronbach's alpha) and test-retest stability methods were employed.

**Results:** Impact score with a score above 1.5 was approved. Content validity index and content validity ratio values of the EIQ tool were 0.970 and 0.940, respectively. The item to total correlation confirmed the construct validity of all seven dimensions of the tool, more than the cut-off (0.3) except lifestyle. Cronbach's alpha coefficient and intra correlation coefficient were acceptable for all dimensions.

**Conclusion:** The Persian version of EIQ is a valid and reliable scale. This tool is valid and reliable for investigating the long-term impact of endometriosis in Iranian society.

**Keywords:** Translation, Psychometrics, Validity, Reliability, Endometriosis impact questionnaire, Endometriosis.

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## P-27

### Metformin effect on serum and follicular fluid hormone levels in women with polycystic ovarian syndrome: An RCT

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**Background:** Polycystic ovary syndrome (PCOS) is one of the most common causes of infertility, usually characterized by anovulation due to hormonal imbalances, including excessive luteinizing hormone (LH), normal level of follicle-stimulating hormone (FSH), insufficient gonadotropin secretion, hyperandrogenism, and the presence of polycystic ovarian morphology. Metformin is the most commonly prescribed medication for managing reproductive issues linked to insulin resistance in PCOS and is widely used to regulate menstrual cycles.

**Objective:** In this study, the effects of metformin on the levels of LH, FSH, testosterone, and estradiol hormones were investigated to predict reproductive function in women with PCOS.

**Materials and Methods:** This experimental study was a randomized, double-blind clinical trial conducted at the Qom University Jihad Infertility Treatment Center, Qom, Iran on 40 women with PCOS who were candidates for intracytoplasmic sperm injection. Participants were selected based on the Rotterdam criteria and ultrasound findings anti-Mullerian hormone levels, LH/FSH ratio, normal thyroid function and

prolactin. Patients with systemic diseases, endocrine disorders or taking drugs affecting metabolism were excluded. Randomization was performed using colored cards (white: placebo, black: metformin). The metformin group (n = 20) received 1500 mg of metformin daily and the placebo group (n = 20) received 25 mg of ORS solution, both for 8 wk. Treatment began 2 months before the ovulation cycle and continued until the day oocyte retrieval. Finally, the levels of LH, FSH, testosterone and estradiol in serum and follicular fluid were measured by enzyme-linked immunosorbent assay.

**Results:** In the metformin group, a significant decrease in the mean serum LH level (p = 0.000) and the mean follicular fluid LH level (p = 0.028) was observed compared with the placebo group. Similarly, the serum FSH level in the metformin group was significantly lower than in the placebo group (p = 0.005). However, the difference in follicular fluid FSH level between the 2 groups was not statistically significant (p = 0.074). Serum estradiol levels did not show a significant difference between the metformin and placebo groups (p = 0.810). In contrast, follicular fluid estradiol levels were significantly reduced in the metformin group compared with the placebo group (p = 0.001). There was no significant difference in serum testosterone levels (p = 0.954) or follicular fluid testosterone levels (p = 0.910).

**Conclusion:** Based on the results of this study, metformin reduced serum and follicular fluid LH levels in PCOS women. Therefore, metformin can reduce the LH to FSH ratio in serum and follicular fluid of these women and improve the quality of oocytes and embryos in PCOS women. In addition, metformin caused an abnormal decrease in androgenic markers, including estradiol and testosterone. Therefore, by reducing androgens, it is possible to improve the follicular growth environment, leading to increased oocyte quality.

**Keywords:** PCOS, Metformin, LH, Testosterone, Estradiol, FSH.

Registration ID in IRCT: IRCT20210210050315N1

## P-28

### Air pollution and gestational diabetes: A cohort study

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**Background:** Gestational diabetes mellitus (GDM) has the potential to negatively impact pregnancy and perinatal outcomes. Furthermore, it can influence the long-term well-being of both the mother and her child. Research indicates that the occurrence of gestational diabetes can be influenced by genetic and environmental factors. One environmental factor, air pollution, may

increase the susceptibility of pregnant women to GDM. Studies suggest that exposure to air pollution could elevate the risk of developing GDM.

**Objective:** This study investigated the relationship between exposures to air pollutants with gestational diabetes.

**Materials and Methods:** This study is a retrospective cohort analysis, utilizing data obtained from a randomized community trial carried out in Iran between September 2016 and January 2019. Pregnant women under 14 wk of gestation who received prenatal care through governmental health services from the first trimester until delivery were considered eligible for enrollment, except those meeting specific exclusion criteria. The study excluded individuals with pre-existing diabetes, under the age of 18, an unknown last menstrual period, absence of ultrasound estimates between 6 and 14 wk of pregnancy, chronic hypertension or asthma, or current use of medications such as oral glucocorticoids, oral  $\beta$ -mimetics,  $\beta$ -blockers, antiretroviral agents, dilantin, or those with a history of bariatric surgery. Additionally, for this analysis, participants lacking data on their residential address or air pollutant exposure during and prior to pregnancy were excluded. Ultimately, a total of 6,090 pregnant women remained eligible for this study. During this study, information on air pollutant levels across 5 cities examined in the initial research was accessible. Each city had a pre-specified protocol for screening all pregnant women for GDM. The first trimester of pregnancy involved early screening of GDM using fasting plasma glucose test from the venous sample, with a specific threshold depending on each screening protocol. Moreover, those who did not have diabetes (overt or gestational) before were screened for GDM again at 24-28 wk of gestation, based on the pre-specified protocol criteria for that city. Concentrations of ozone, nitric oxide, nitrogen dioxide, nitrogen oxides, sulfur dioxide, carbon monoxide, particulate matter < 2.5 or < 10  $\mu\text{m}$  were obtained from air pollution monitoring stations. Pollutant data were available in each city at different intervals and on an hourly basis each day (24 records for 24 hr per day). Using these data in the first stage of analysis, daily mean concentrations were calculated for each air pollutant in each city. Exposure to air pollutants during the 3 months preceding pregnancy and the first, second, and third trimesters of pregnancy for each participant was estimated.

**Results:** The event of GDM was observed in 1144 women out of 6090 participants. None of the logistic regression models showed any statistically significant relationship between the exposure to any of the pollutants and GDM at different time points (before pregnancy, in the first, second and third trimesters of pregnancy and 12 months in total) ( $p > 0.05$ ). Also, none of the adjusted logistic regression models showed any significant association between PM10 exposure and GDM risk at all different time points after adjusting for various confounders ( $p > 0.05$ ).

**Conclusion:** This study did not identify any association between the risk of GDM and exposure to various air pollutants before or during different trimesters of pregnancy. However, these findings should be interpreted with caution due to the limitations in accounting for all potential confounding factors. Further comprehensive cohort studies that address these confounders are urgently needed to better understand the impact of air pollution on GDM.

**Keywords:** Gestational diabetes, Pollutant, Air pollution, PM2.5, PM10.

## P-29 Factors influencing professional communication and teamwork between midwives and physicians

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**Background:** Interprofessional communication in healthcare, particularly in obstetrics, plays a crucial role in ensuring patient safety and improving service quality. Effective and coordinated collaboration between obstetricians and midwives through clear and respectful communication can significantly impact treatment outcomes and patient experience. The World Health Organization has also identified interprofessional education and collaboration as a key strategy for providing safe, patient-centered care.

**Objective:** The primary aim of this study is to examine the factors influencing professional communication and teamwork between midwives and physicians in obstetrics.

**Materials and Methods:** This study was conducted as a systematic scoping review based on PRISMA guidelines. By systematically searching PubMed, SID, CIVILICA, and Magiran databases (2014-2024) using the combined keywords "interprofessional communication", "midwife or physician", and "obstetrics and gynecology", 45 articles were selected for full-text evaluation from among the initial 367 studies, after removing duplicate articles and screening the title and abstract. Finally, 10 studies that met the inclusion criteria including publication in English/Persian, focusing on midwife-physician collaboration, and relevance to the field of obstetrics and gynecology were included in the study. Data related to communication facilitators and barriers were examined using thematic analysis.

**Results:** This study showed that communication facilitators between doctors and midwives include effective communication skills such as active listening, clear communication, and mutual respect. Additionally,

trust and mutual respect, communication skills training, empathy, creating a supportive environment, team coordination, and proper task division are factors that improve collaboration and quick decision-making. On the other hand, communication barriers include cultural differences, work pressure, resistance to change, lack of team coordination, and unclear task division, which can reduce the quality of communication and negatively affect effective collaboration.

**Conclusion:** The results of this study indicate that effective communication and the development of teamwork skills are fundamental to fostering collaborative relationships. Individuals who clearly understand their professional and team responsibilities, and recognize the roles of other team members, can contribute more effectively to team activities. This enhances the utilization of the capabilities of other team members and improves overall team performance.

**Keywords:** Professional communication, Midwife, Physician, Influencing factors.

### P-30

#### Explanation of mothers regarding the challenges of fetal anomaly screening tests: A qualitative study

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**Background:** Nowadays, the implementation of fetal screening tests has become an integral part of prenatal care worldwide. Although the application of these tests has led to a reduction in the incidence of births with chromosomal abnormalities, their execution may result in psychological consequences for women.

**Objective:** Therefore, this qualitative study was designed to elucidate and describe the profound concerns of pregnant and postpartum women regarding the situations and challenges associated with the performance of screening tests for fetal anomalies.

**Materials and Methods:** The present study is a qualitative study utilizing a conventional content analysis approach. This study was conducted in the second half of 2023 in the comprehensive health service centers of Khalkhal County. Data were collected through semi-structured in-depth interviews with 16 pregnant mothers and 8 postpartum mothers who had undergone fetal anomaly screening tests. All interviews were audio-recorded and transcribed, with data analysis carried out using MAXQDA software, version 20.

**Results:** In this study, participants' perceptions of the challenges associated with prenatal screening tests

encompassed 7 categories and 12 subcategories. The categories included the creation of psychologically hazardous, stressful conditions and the bitter experience of pregnancy; challenges in decision-making regarding the sequential testing process; challenges in decision-making concerning future childbearing; financial challenges; challenges related to laboratory centers; challenges in decision-making regarding abortion; and challenges associated with a lack of support and assistance.

**Conclusion:** Fetal health screening tests can assist in identifying women at risk of having offspring with chromosomal disorders. The widespread implementation of prenatal screening tests significantly reduces the incidence of these conditions. However, certain limitations and challenges associated with fetal screening tests have introduced ethical and legal considerations, complicating the necessity for comprehensive and extensive application of these tests.

**Keywords:** Prenatal screening, Fetal abnormalities, Challenges, Pregnant women.

### P-31

#### The effect of a period of combined exercise training in overweight pregnant women on interleukin-10 serum levels

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**Background:** Overweight and obesity during pregnancy increase health risks for both mother and baby, including inflammation and metabolic issues. Interleukin (IL)-10, an anti-inflammatory cytokine, affects pregnancy outcomes. While combined aerobic and resistance training improves metabolic markers in non-pregnant individuals, its impact on IL-10 in pregnancy is unclear. This study examines how combined exercise influences IL-10 levels in overweight pregnant women.

**Objective:** To assess the impact of a combined exercise program on IL-10 serum levels in overweight pregnant women.

**Materials and Methods:** In this randomized clinical trial, 25 overweight pregnant women (> 25 pre-pregnancy body mass index < 30 kg/m<sup>2</sup>) who met the inclusion criteria attending the prenatal clinic of Sarem hospital in Tehran, Iran in 2019-2020 were selected. The participants were randomly assigned to either an intervention group (n = 13) or a control group (n = 12). The exercise program for the intervention group consisted of 5 days/wk of a combination of aerobic, resistance, and stretching exercises from the 16<sup>th</sup>-18<sup>th</sup> wk of pregnancy to the 36<sup>th</sup>-37<sup>th</sup> wk of pregnancy. The participants in the intervention group recorded their exercise routine daily on forms provided to them. The control group continued their usual daily activities until the end of pregnancy. Both groups went through routine prenatal care. Both groups were evaluated for blood

pressure, weight, and anthropometric characteristics during the 16-18, 25-29, and 36-40 wk of pregnancy, and venous blood samples were collected.

**Results:** Both before intervention at 16-18 wk, and after intervention at 25-29 wk of gestation, no statistically significant difference was shown between groups regarding serum IL-10 levels, but after intervention at 36-40 wk of gestation this difference was significant, ( $15.76 \pm 14.48$  vs.  $32.54 \pm 37.62$  pg/ml,  $p = 0.043$ ). Based on the results obtained from linear regression analysis adjusting for the effect of various effective factors on IL-10 levels (such as maternal age and BMI) at both evaluations after intervention at 25-29, and 36-40 wk of gestation, IL-10 levels were found to be significantly lower in the intervention group compared to the control group ( $\beta$ : -0.428,  $p$ : 0.04,  $\beta$ : -0.36,  $p$ : 0.01 respectively).

**Conclusion:** The study found that combined exercise training significantly influences serum IL-10 levels in overweight pregnant women, especially between 36-40 wk of pregnancy. This suggests that exercise may help manage inflammation, potentially improving maternal and fetal health. Further research is needed to understand the long-term effects of these findings.

**Keywords:** Combined exercise, Pregnancy, Interleukin-10, Overweight.

**Registration ID in ICT:** ICT20190410043222N1

## P-32

### The importance of mental health nursing in infertile couples

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**Background:** Infertility is defined as the inability to conceive after 12 months of regular unprotected intercourse. It can have negative physical, emotional, and social impacts on couples. To avoid this stress and life crisis, individuals often seek alternative treatment options, with assisted reproductive technology frequently considered a solution. However, this alternative can also be a significant source of stress. Daily injections, semen analyses, multiple ultrasounds, lengthy checklists, and financial costs have repeatedly been reported by individuals undergoing infertility treatment as psychological stressors.

**Objective:** The present study was conducted to examine the significance of psychiatric nursing and the role of psychiatric care in the management of infertility among couples.

**Materials and Methods:** In this type of systemic study, the keywords; Stress, Anxiety, Infertility, Assisted Reproductive Technology, and Nursing Care were systematically searched in English language in the online databases PubMed, Google Scholar, and Scopus within the time frame of 2015-2024. My criteria for

inclusion and exclusion of articles is being international and focusing on psychological discussions.

**Results:** Women undergoing infertility treatment bear a significant burden of physical and psychological fatigue. In addition to medical care, they require human and emotional support. The heightened sensitivity of these women to the emotions and changes arising from infertility underscores the importance of freely expressing these feelings. To achieve this goal, nursing interventions and positive interactions with participants are essential. Nurses can play a pivotal role in reducing participants stress and anxiety and addressing their emotional needs by providing specialized care. Emotional support, delivering accurate information and necessary education, respecting participants values, and establishing a warm and effective communication are key actions in this regard. Therapeutic communication is recognized as one of the effective methods for reducing anxiety and holds particular significance in nursing care. The therapeutic communication techniques employed by nurses can lead to positive changes in the psychological and clinical status of participants.

**Conclusion:** Therefore, nursing counseling should be planned from the beginning of the infertility treatment process and continue until its conclusion, regardless of the outcome.

**Keywords:** Stress, Anxiety, Infertility, Assisted reproductive technology, Nursing care.

## P-33

### Global, regional, and national prevalence and disability-adjusted life years for secondary infertility in Iran and provinces of Iran: Results from the global burden of disease study, 1990-2021

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**Background:** Secondary infertility is defined as the inability to conceive or carry a baby to term after previously giving birth, represents a significant global reproductive health challenge.

**Objective:** This study aimed to comprehensively analyze the global, regional, national (Iran), and provincial burden of secondary infertility from 1990-2021, including point prevalence and years lived with disability.

**Materials and Methods:** We conducted a cross-sectional analysis using Global Burden of Disease 2021 data. Age-standardized point prevalence and year of healthy life lost due to disability rates (per 100,000 populations) with 95% confidence intervals were calculated for benchmark years (1990 and 2021) at all geographical levels, including Iran's 32 provinces. Statistical modeling was employed to estimate trends.

**Results:** Globally, age-standardized point prevalence increased from 2429.8 (95% CI: 1418.7-4097.3) in 1990-2860. 8 (1686.1-4801.1) in 2021 for women, and from 826.6 (443.6-1453.6) to 939.9 (493.4-1672.0) for men. In Iran, prevalence remained stable (women: 1141.6-1174.5; men: 434.6-435.7). Provincial disparities emerged, with highest prevalence in Tehran, Mazandaran, and Alborz (women) and Khorasan-e-Razavi, Khuzestan, and South Khorasan (men). Years lived with disability rates were lower in Iran (4.1; 1.3-9.8) than globally (9.7; 3.4-24.8).

**Conclusion:** While global secondary infertility burden increased, Iran maintained lower prevalence and year of healthy life lost due to disability rates than global and Middle East and North Africa averages. Provincial-level variations highlight potential healthcare disparities requiring targeted interventions.

**Keywords:** Infertility, Global burden of disease, Prevalence, Years lived with disability.

### P-34

#### The association between polycystic ovary syndrome and vaginal microbiome disorders

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Polycystic ovary syndrome (PCOS) is an endocrine disease that affects women of reproductive age. This disease is one of the causes of infertility. Changes in the vaginal microbiome appear to be one of the causes of PCOS. This study aimed to review the impact of the vaginal microbiome on polycystic ovary disease. In this narrative review study, articles were searched using the keywords "vaginal microbiomes", "infertility", "polycystic ovary syndrome", "reproductive health", and "vaginitsis" through Pub-Med, Google Scholar, and Scopus databases, and the most relevant articles published since 2020 were reviewed. The inclusion criteria for studies included full-text articles, publication in Persian or English, and descriptive, analytical, and experimental studies. The search resulted in 56 articles, and the results were derived from 13 related studies that had full text. A review of studies has shown that disruptions in the vaginal and cervical microbiomes are associated with gynecological diseases including endometriosis, PCOS, and infertility. The microbiome plays an important role in maintaining the body's physiological balance. Changes in the microbiome can lead to changes in the body. Estrogen deficiency occurs in PCOS, which leads to disruptions in the vaginal microbiome, and these changes in the microbiome can also lead to PCOS. Alteration of the vaginal microbiome can lead to PCOS and ultimately infertility. Therefore,

lifestyle modifications, including hygiene, use of probiotics, and treatment of vaginal infections, are recommended in the management of PCOS.

**Keywords:** Life style, Infertility, Polycystic ovary syndrome, Reproductive health, Vaginitis.

### P-35

#### The impact of the BETTER model on sexual aspects of infertility: A narrative review

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Infertility is one of the major challenges, which affects the psychological, social, and sexual aspects of couples. Sexual problems, as a critical aspect of quality of life, are more prevalent in infertile couples which require special attention. The bring up, explain, tell, timing, educate, and record (BETTER) model has been introduced as a comprehensive counseling approach to improve sexual quality of life and reduce infertility-related stress. This study aimed to evaluate the impact of the BETTER model on various sexual aspects of infertile couples' lives. In this narrative review study, relevant Persian and English articles were collected from Google Scholar, PubMed, Scopus, and Cochrane databases. Keywords such as "infertility", "counseling", "BETTER Model", and "sexual" were reviewed without a time frame. After an initial review, 187 articles were obtained, and finally 7 articles were further reviewed. The use of the BETTER model has a significant effect on improving sexual and psychological problems in infertile women, including: improved sexual function, reduced anxiety and psychological stress, Increased marital satisfaction, and improved quality of sexual life. However, limitations such as the lack of long-term follow-up, focus on infertile women, and the lack of joint studies in couples should be further investigated. The BETTER model has a role as a comprehensive model in improving sexual aspects in infertile women. Incorporating this model into infertility counseling programs can be a step towards improving the mental health and quality of sexual life.

**Keywords:** Infertility, BETTER model, Sexual counseling, Sexual function, Marital satisfaction.

### P-36

#### The importance of psychological and social support for infertile women

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Infertility is a difficult condition that has a significant psychological impact on women. Providing adequate psychological and social support can reduce psychological problems in infertile women. The purpose of the present study was to assess the importance of psychological and social support for infertile women. In this narrative review study, articles were searched using the keywords "infertility", "social support" and "psychological support" through Web of Science, SID, Scopus, and PubMed and Google Scholar database, and the most relevant articles published from 2020-2025 were reviewed. The inclusion criteria for studies included full-text articles, publication in Persian or English, and descriptive, analytical, and experimental studies. The articles were reviewed by 2 people and screened based on title and abstract, and the quality of the articles was assessed using the CASP and STROBE tools. The results were derived from 6 related studies that had full text. A review of studies showed that when women have family support and higher levels of education, they are less anxious about their infertility situation. A person with a social network is better off when facing adversity than a person without support or without a support network ( $p < 0.05$ ). Social and psychological support is effective on the quality of reproductive life. With an increase in the level of social support, symptoms of anxiety, depression, and negative self-image in couples receiving infertility treatment decreased. By increasing the level of social and psychological support received by women undergoing infertility treatment, symptoms of depression, stress, negative self-concept, and worry about infertility are reduced, and it has a positive effect on the quality of reproductive life.

**Keywords:** Female infertility, Psychosocial support, Social support.

### P-37

#### The impact of logotherapy on acceptance and self-awareness in the mental health of infertile couples: A systematic review

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**Background:** Infertility is a significant global health issue that presents serious challenges for families. A diagnosis of infertility can lead to stressful experiences and negative emotional reactions, such as anger and isolation, which can impact individuals' mental health. Acceptance of this condition is essential for those facing

infertility, and developing self-awareness can facilitate this process.

**Objective:** This study investigated the impact of meaning therapy on acceptance and self-awareness in the mental health of infertile couples.

**Materials and Methods:** This study employs a systematic review methodology. It involved a comprehensive search using keywords from the phrases "Logotherapy", "acceptance", and "self-awareness" across reliable databases such as SID, Magiran, Google Scholar, and PubMed. The review focused on literature published from 2015-2023. The included inputs comprised quantitative studies, qualitative studies, mixed methods, conference papers, theoretical articles, experimental studies, protocols, and discussions or commentaries related to factors affecting acceptance and self-awareness in the mental health of infertile couples. 2 researchers independently selected a total of 120 articles for review. Studies that were not relevant to the topic were excluded based on analysis and inclusion criteria. After searching and eliminating duplicate articles through objective screening, a total of 98 articles were reviewed, of which 63 were ultimately selected for the study.

**Results:** The studies indicate a valuable consensus among experts regarding the integration of meaning therapy in addressing infertility and enhancing the mental health of couples. Meaning therapy plays a critical role in fostering acceptance and self-awareness among infertile patients. Given the numerous psychological challenges faced by infertile individuals, it is essential to explore intervention methods that can help alleviate these issues. Enhancing acceptance of negative emotions can reduce stress and improve overall quality of life, enabling couples to better understand each other's feelings and needs. Self-awareness, self-esteem, and self-confidence are fundamental indicators of an individual's strengths and weaknesses. Increased self-awareness can lead to improved communication between partners and heightened empathy. Furthermore, since infertility can affect individuals throughout their lives, it necessitates greater attention and support from family, medical staff, and society to promote adaptability and enhance quality of life. Thus, the findings underscore the need for comprehensive training programs for nurses to equip them with the necessary skills in meaningful therapy for the care of infertile individuals.

**Conclusion:** Meaning therapy positively impacts the mental health of couples, particularly in fostering acceptance and self-awareness among infertile individuals. Given its beneficial effects observed in this and other studies, it is advisable for service providers and managers to incorporate this method into treatment approaches aimed at enhancing acceptance and self-awareness in various therapeutic contexts.

**Keywords:** Logotherapy, Acceptance, Self-awareness, Infertility.

### P-38

#### The role of artificial intelligence in the care of participants with infertility: A systematic review

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**Background:** Infertility is a significant reproductive health issue affecting millions of couples worldwide. Despite advances in medical science, more than half of infertility cases remain unexplained, highlighting the need for innovative diagnostic and treatment models. Current treatment options often involve substantial financial and diagnostic challenges, contributing to a significant psychological burden for those affected. Therefore, there is a pressing need for new tools to enhance infertility care. Artificial intelligence (AI) is emerging as a transformative force, offering great potential to improve medical care, particularly in the field of infertility.

**Objective:** This study aims to investigate the role of AI in the care of participants experiencing infertility.

**Materials and Methods:** This study employed a systematic and prospective review methodology. It involved a comprehensive search using the keywords "artificial intelligence", "infertility", and "patient care" across reliable databases such as Scopus, ISI, SID, Magiran, Google Scholar, and PubMed. The review focused on literature published from 2015-2023. The included entries comprised quantitative, qualitative, and mixed-methods studies, as well as articles, reviews, experimental studies, protocols, and discussions related to the role of AI in the care of patients with infertility. 2 independent researchers selected a total of 80 articles for review. Studies deemed irrelevant to the topic were excluded based on analysis and inclusion criteria. After searching and eliminating duplicate articles through objective screening, a total of 50 articles were reviewed, of which 30 were ultimately selected for the study.

**Results:** The study's results indicate that experts foresee valuable insights regarding the integration of AI into infertility care. AI is defined as the capability of engineered systems to learn, process, and analyze data. The incorporation of AI into the clinical management of infertility represents a transformative and essential approach for optimizing treatment outcomes, enhancing assisted reproductive technologies, and improving participant's communication. AI can analyze clinical and laboratory data to identify factors contributing to infertility, assess sperm and egg quality, and select the most effective fertility treatment options. This capability allows for a more personalized approach and enables healthcare providers to engage in meaningful discussions with participants about the feasibility and likelihood of clinical interventions. Furthermore, AI can aid in counseling and providing accurate information to

participants, which can enhance awareness, reduce stress, optimize diagnostic and treatment processes, and decrease the time and costs associated with participant's care.

**Conclusion:** Artificial intelligence holds significant potential to enhance the diagnosis and treatment of infertility, as well as overall participant's care. Given the recent advancements in AI technologies, it is anticipated that these tools will increasingly be utilized in clinical settings, improving treatment outcomes and the quality of life for participants. However, to fully leverage this technology, further research, improved data quality, and careful consideration of ethical and privacy issues are necessary. This systematic review demonstrates that the integration of artificial intelligence in infertility management can contribute to improved clinical outcomes and enhance participant's quality of life.

**Keywords:** Artificial intelligence, Infertility, Patient care.

### P-39

#### Geomorphological influences on gut microbiome and human fertility: An integrative perspective

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Geomorphology, the scientific discipline examining landforms and geological processes, exhibits a profound interconnection with human health. Recent investigative research demonstrates that geomorphological variations significantly impact gut microbiome composition and functionality, which play a critical role in health and disease mechanisms. This comprehensive study explores the intricate relationships between geomorphological factors, gut microbiome dynamics, and consequential health outcomes, with specific emphasis on fertility challenges. This analytical review study was based on 21 articles published from 2018 onwards. Environmental topographical characteristics fundamentally influence water and soil quality, subsequently affecting agricultural productivity, ecosystem diversity, and nutritional environments. Variations in elevation, soil composition, and environmental conditions create unique microbial landscapes that potentially modulate gut microbiome diversity. Empirical evidence, substantiates that populations inhabiting different geographical elevations demonstrate distinctly different gut microbiome profiles, potentially attributable to adaptive microbial responses to specific environmental conditions like oxygen pressure, temperature variations, and dietary compositions. Gut microbiome disruptions are increasingly recognized as significant contributors to reproductive health challenges. Intestinal dysbiosis can

precipitate chronic inflammatory responses, oxidative stress, and metabolic alterations that directly impact reproductive functionality. Research by Wang et al. provides compelling evidence of reduced microbial diversity and increased pathogenic bacterial presence in infertile women, underscoring the critical importance of maintaining optimal gut microbiome equilibrium for reproductive health. Conclusively, geomorphological factors exert substantial influences on human health through intricate gut microbiome modulation mechanisms. Enhanced understanding of these complex interrelationships promises innovative approaches to public health improvement and potential fertility interventions. While current research provides promising insights, further comprehensive investigations are imperative to delineate underlying mechanisms and explore practical applications. Future research should prioritize multidisciplinary approaches, integrating geomorphological, microbiological, and reproductive health perspectives to develop more nuanced, targeted interventional strategies.

**Keywords:** *Gastrointestinal microbiome, Environmental medicine, Geomorphology.*

#### P-40

### **Protective role of naringenin in mitigating steroidogenesis disturbances in human granulosa cells from normal and polycystic ovaries**

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**Background:** Estradiol and progesterone are the primary steroid hormones that may be crucial throughout the follicular and luteal stages of the menstrual cycle, oocyte fertilization, and embryo implantation. The granulosa cells (GCs) of patients with polycystic ovarian syndrome (PCOS) do not enhance the expression of aromatase and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD), resulting in significantly reduced release of estrogen and progesterone. Naringenin, a principal constituent of grapefruit, has been documented to function as a modulator of some reproductive pathways.

**Objective:** This study evaluated the role of naringenin in regulating the endocrine activity of granulosa cells in women with PCOS and those without.

**Materials and Methods:** This experimental study was conducted on ovarian GCs obtained from 40 women who visited Shiraz Ghadir Mother and Child Hospital, Shiraz, Iran for in vitro fertilization or intracytoplasmic sperm injection between November 2023 and August 2024. The control group (N-GCs) included 20 women with normal ovarian function, diagnosed primarily with

male factor infertility, tubal pathology, unexplained infertility, or those participating as egg donors or having a normal ovarian reserve. The case group (PCO-GCs) consisted of 20 women diagnosed with PCOS based on the Rotterdam criteria, as determined by a gynecologist. Eligible participants were women aged between 18 and 35 with a body mass index ranging from 18.5-30 kg/m<sup>2</sup>. Individuals were excluded if they had significant medical conditions such as other causes of hyperandrogenism, thyroid disorders, endometriosis, chronic metabolic syndrome, or diabetes, or if they reported cigarette smoking or alcohol consumption. The GCs were obtained from dominant follicles using follicular aspirates on the day of oocyte extraction. The GCs were cultivated in Dulbecco's modified Eagle's medium/F12 media. To investigate the impact of naringenin on aromatase and 3 $\beta$ -HSD activities, GCs (4 $\times$ 10<sup>6</sup> cells) were separated into 2 groups and cultivated in 6-well plates for 48 hr, with or without naringenin (200  $\mu$ M). The media were collected to assess estradiol and progesterone as markers of aromatase and 3 $\beta$ -HSD activity.

**Results:** The basal estradiol ( $p = 0.008$ ) and progesterone ( $p = 0.013$ ) levels in the PCOS group were considerably lower than in the normal group. Naringenin-treated GCs exhibited a significant increase ( $p < 0.001$ ) in total estradiol and progesterone release after 48 hr compared to untreated GCs. Nonetheless, naringenin therapy did not restore sex hormone production to normal levels.

**Conclusion:** Our findings indicated that ovarian aromatase and 3 $\beta$ -HSD activities in PCOS were diminished compared to healthy women. An enhancement of aromatase and 3 $\beta$ -HSD activities marks the stimulatory effect of naringenin. This influence on steroidogenic enzyme activity suggests a post-transcriptional modulation of these enzymes by naringenin.

**Keywords:** *Estradiol, Naringenin, Progesterone, PCOS, Granulosa cells.*

#### P-41

### **Ameliorative effects of lycopene on sperm biochemical parameters during cryopreservation in the asthenozoospermic men**

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**Background:** Asthenozoospermia is considered one of the most prevalent causes of male infertility. In asthenozoospermia cases where enough suitable sperm cannot be provided for assisted reproductive technology or where it is impossible to collect fresh semen from the men on the day of assisted reproductive technology for any reason, cryopreservation can help to recover sperm and oocyte at the same time. Cryopreservation of human

semen increases reactive oxygen species, leading to oxidative damage to the sperm. Therefore, adding an antioxidant to the freezing medium could be useful. Lycopene, as a red carotenoid and powerful antioxidant, has many positive effects on sperm biochemical parameters.

**Objective:** This experimental study aims to assess the effect of lycopene supplementation on sperm biochemical parameters during cryopreservation in asthenozoospermia men.

**Materials and Methods:** Semen samples were collected from 30 asthenozoospermic men in 1403 at Mehr Infertility Treatment Center, Rasht, Iran. Each sample was then divided into 3 groups (n<sub>1</sub>, n<sub>2</sub>, n<sub>3</sub>): n<sub>1</sub>: control (fresh), n<sub>2</sub>: freeze (treated with cryoprotectant alone), and n<sub>3</sub>: freeze + lycopene (treated with cryoprotectant and 5 µmol/L lycopene). 30 samples were examined in each group. In the freezing groups, samples were cryopreserved with human sperm freezing medium and rapid freezing method. In each sample, using an enzyme-linked immunosorbent assay, the level of sperm antioxidants, including catalase, glutathione, and superoxide dismutase, and the level of sperm malondialdehyde (MDA) were investigated.

**Results:** There was a significant decrease in the mean levels of sperm antioxidants in the freeze group compared to the control counterpart ( $p < 0.001$ ). At the same time, there was a significant increase in the mean sperm levels of MDA ( $p < 0.001$ ). Moreover, in the freeze+ lycopene group, the mean levels of antioxidants increased significantly ( $p < 0.001$ ), while the mean levels of MDA decreased significantly compared to the Freeze group ( $p < 0.001$ ).

**Conclusion:** Given our findings, lycopene ameliorates the adverse effects of cryopreservation on sperm quality in asthenozoospermic men by reducing reactive oxygen species production and preventing its undesired effects on sperm function by increasing sperm antioxidants, and reducing MDA levels.

**Keywords:** Asthenozoospermia, Cryopreservation, Biochemical parameters, Oxidative stress, Lycopene.

## P-42

### Evaluation of the effect of human testicular cell conditioned media on the in vitro development of follicles from cryopreserved human ovarian cortical pieces: A potential approach for fertility preservation

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**Background:** Ovarian tissue (OT) cryopreservation is a method for fertility preservation in female cancers, and after warming, the current standard approach for its use is OT transplantation. However, this cannot be an option in hematologic cases, due to the high risk of cancer reimplantation after remission. In vitro growth of isolated follicles is an alternative for OT transplantation.

**Objective:** The aim was to evaluate the effects of the human testicular cell conditioned medium (HTCCM) on the in vitro growth of isolated follicles, after 2 methods of ovarian cryopreservation: vitrification and slow freezing.

**Materials and Methods:** In this experimental study, human ovarian cortical strips from 8 cancer patients who were candidates for fertility preservation at our institute were frozen and then cultured in a multiple-step system, after thawing. The culture media were a routine culture medium or a medium containing HTCCM. Folliculogenesis, the proportion of morphologically normal follicles, follicle diameter, hormone production, and gene expressions (*PTEN*, *BMP-15*, *PDGF*, *GDF-9*, and *GAPDH*) were analyzed during 21 days of culture.

**Results:** Follicular morphology was the same after the 2 cryopreservation methods, and decreased during the organ culture ( $p < 0.001$ ). However, while follicular growth occurred independently in the slow-freezing group, folliculogenesis was activated in the vitrified group in the presence of HTCCM ( $p = 0.081$ ). There were no significant differences in the estradiol or progesterone secretions, either in the presence or absence of HTCCM ( $p = 0.530$ ). The secreted hormonal levels didn't differ between the 2 cryopreserved groups ( $p = 0.640$ ). In addition, the folliculogenesis gene expressions didn't show significant differences between the cultured groups, except for BMP-15, in the slow/HTCCM ( $p < 0.001$ ). Follicle diameter was higher in the slow/HTCCM, only compared to vitrification culture in the absence of HTCCM ( $p = 0.023$ ).

**Conclusion:** Although HTCCM did not affect the follicular morphology or hormone secretion, it can compensate for the observed delay in ovarian folliculogenesis, as it could promote gene expression in the ovarian in vitro culture.

**Keywords:** Fertility preservation, Ovarian culture, Follicle culture, Testicular interstitial cell, Conditioned medium, Folliculogenesis.

## P-43

### Papaverine ameliorates sperm biochemical parameters and oxidative stress in asthenozoospermic men during cryopreservation

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**Background:** Asthenozoospermia, characterized by reduced or complete loss of sperm motility, is a prevalent cause of male infertility. Sperm cryopreservation, vital for assisted reproductive technologies, induces cryoinjury, leading to structural and functional sperm impairment through elevated oxidative stress, DNA fragmentation, and apoptosis. Excessive reactive oxygen species (ROS) generation during freeze-thaw cycles heightens oxidative damage, particularly in asthenozoospermic men, reducing fertility potential. Papaverine (PPV), a powerful antioxidant, effectively reduces oxidative damage, with prior studies showing its protective effects on cryopreserved normozoospermic men.

**Objective:** In this study, the effects of PPV on sperm biochemical factors and intracellular ROS levels were investigated in asthenozoospermic men during cryopreservation.

**Materials and Methods:** Semen samples from 30 asthenozoospermic men, obtained at the Amir-AL-Momenin Infertility Treatment Center, Arak, Iran, between November 2023 and May 2024, were examined in this experimental in vitro study. Each sample was divided into 3 groups: control (fresh semen), freeze (cryoprotectant only), and freeze + PPV (cryoprotectant + 100  $\mu$ M PPV). Cryopreservation involved a standardized sperm freezing medium and a rapid vitrification technique. Sperm malondialdehyde (MDA), total antioxidant capacity (TAC), and antioxidant enzymes catalase, glutathione, superoxide dismutase, were measured via enzyme-linked immunosorbent assay. Intracellular ROS levels were assessed using the DCFH-DA fluorescent probe (Kiazist ROS Assay Kit, Iran) with a partec pas flow cytometer. Data were analyzed using FlowJo™ software, and results were expressed as mean fluorescence intensity.

**Results:** The freeze group exhibited significantly reduced mean levels of glutathione, catalase, superoxide dismutase and TAC (all  $p < 0.001$ ) and elevated mean MDA and ROS levels ( $p < 0.001$ ) compared to the control group. Conversely, the freeze + PPV group demonstrated significantly enhanced antioxidant enzyme levels and TAC ( $p < 0.001$  for all), along with reduced MDA ( $p < 0.001$ ) and ROS ( $p = 0.040$ ) levels, compared to the freeze group.

**Conclusion:** This study demonstrated that the addition of PPV to the sperm freezing medium effectively reduces oxidative stress and intracellular ROS levels in cryopreserved sperm in asthenozoospermic men. These findings suggest that PPV has the potential to mitigate freeze-thaw-induced damage and improve outcomes in assisted reproductive technology.

**Keywords:** Papaverine, Asthenozoospermia, Sperm cryopreservation, Oxidative damage.

#### P-44

### Supplementation of freezing media with zinc and melatonin improves the sperm functional parameters in asthenozoospermic men

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**Background:** Decreased sperm motility, viability, and fertilization potential have been reported following the freezing and thawing process. In this regard, to enhance human sperm resistance to the cryopreservation stress a variety of cryoprotective substances have been proposed.

**Objective:** The present study aimed to assess the effects of zinc and melatonin supplementation to freezing medium on spermatozoa in asthenozoospermic men.

**Materials and Methods:** In this experimental study, 10 semen samples of asthenozoospermic men attending the Infertility Research and Treatment Center of ACECR Khuzestan, Ahvaz, Iran from September 2023 to October 2024 were prepared and split into 2 equal aliquots. Smokers, alcohol consumers and men with history of varicocele, genital tract infection, chronic illness and serious systemic diseases were excluded from the study. A freezing medium containing 50 mM ZnSO<sub>4</sub> and 1 mM melatonin were mixed with aliquots and then treated samples were cryopreserved using the slow freezing protocol in liquid nitrogen. Samples were thawed and washed after 2 wk and then sperm viability, motility and functional tests were analyzed.

**Results:** Our study showed that the addition of zinc and melatonin to the freezing medium after thawing significantly improves sperm motility and viability. Also, compared to the zinc and melatonin-free group membrane the acrosomal integrity of human sperm were higher after thawing.

**Conclusion:** Our results showed that the addition of zinc and melatonin to the freezing medium could improve post-thaw quality of cryopreserved human sperm.

**Keywords:** Cryopreservation, Zinc, Melatonin, Spermatozoa.

#### P-45

### Optimizing assisted reproductive technologies: Evaluating the role of polyvinylpyrrolidone, hyaluronic acid, and thymoquinone in sperm immobilization and embryo development

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Poly Vinyl Pyrrolidone (PVP) is a commonly utilized synthetic polymer known for its biocompatibility. It plays an important role in assisted reproductive

technologies, especially in the process of intracytoplasmic sperm injection (ICSI). PVP helps to immobilize sperm and safeguards oocytes during the microinjection process, enhancing the chances of successful fertilization and the development of embryos. The aim of this assessment is to explore the functions of PVP, hyaluronic acid (HA), and thymoquinone (TQ) in the immobilization of sperm and the quality of embryos, with the intention of enhancing the results of assisted reproductive technologies. A comprehensive narrative review was carried out utilizing data gathered from PubMed, Google Scholar, and Science Direct, with the cutoff date being November 2024. The search terms employed included "PVP", "ICSI", "Hyaluronic acid", "Thymoquinone", and "sperm immobilization". Original research articles and review papers that examined the impact of these compounds on fertilization and embryo quality were considered for inclusion. Studies that were not in English or did not undergo peer review were omitted from the analysis. Research indicates that incorporating PVP into microinjection solutions can improve embryo growth; however, high levels or extended exposure may negatively affect sperm DNA integrity. On the other hand, HA presents a more natural and safer option, enhancing fertilization rates and embryo quality by favoring mature sperm with intact DNA. Additionally, TQ, a substance extracted from *Nigella sativa*, shows promise in effectively immobilizing sperm with reduced toxicity, though its clinical use remains less widespread than that of HA. PVP remains an important part of ICSI procedures, but HA is gaining recognition as a better choice because of its safety and efficiency in selecting sperm and promoting embryo growth. Although TQ shows promise, it needs more testing to confirm its effectiveness.

**Keywords:** Poly vinyl pyrrolidone, Intracytoplasmic sperm injection, Hyaluronic acid, Thymoquinone, Sperm immobilization, Embryo quality.

#### P-46

### Synthesis, characterization and investigation of the effects of soy protein nanoparticles containing sambucus nigra extract on the human sperm parameters after freezing: In vitro study

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**Background:** Cryopreservation followed by thawing is the standard method for preserving male fertility, though

it adversely affects sperm quality by impairing motility, morphology, and overall functionality.

**Objective:** This study aimed to synthesize and characterize soy protein nanoparticles (SPN) loaded with *Sambucus nigra* extract (*S.nigra*) and evaluate their effects on human sperm parameters following cryopreservation.

**Materials and Methods:** This experimental in vitro study was conducted in 2023 at Shahid Sadoughi University of Medical Sciences, Yazd, Iran. SPN and SPN containing *S.nigra* extract (*S.nigra*-SPN) were synthesized and characterized. Sperm samples from healthy donors were divided into 5 groups: control (before freezing); control, SPN, *S.nigra*, and *S.nigra*-SPN (after freezing). The treated groups were incubated for 2 hr at room temperature prior to rapid freezing. 20 samples were analyzed for post-thaw sperm motility, morphology (diff-quick staining), and chromatin integrity.

**Results:** Encapsulation efficiency and loading capacity were 90% and 36.16%, respectively, and characterization confirmed successful nanoparticle synthesis. The *S.nigra* extract was released in a controlled manner, and both blood compatibility and biocompatibility were verified. Post-thaw analysis revealed a significant decline in sperm parameters across all groups ( $p < 0.05$ ); however, those treated with *S.nigra*, SPN, and *S.nigra*-SPN showed improved outcomes compared to the controls. Among them, the *S.nigra*-SPN group demonstrated the most notable improvements in motility and morphology ( $p \leq 0.001$ ). Additionally, DNA fragmentation ( $p = 0.07$ ) and chromatin damage ( $p = 0.28$ ) were reduced insignificant in the nanoparticle-treated groups.

**Conclusion:** SPN enhances post-thaw sperm quality, stabilizes *S.nigra* extract delivery, and synergizes effects, aiding assisted fertilization outcomes.

**Keywords:** Spermatozoa, Cryopreservation, *Sambucus nigra*, Nanoparticles, Soy protein.

#### P-47

### Study on the sensitization of sexual cycle to circadian light-dark conditions and the ameliorative effect of melatonin in Wistar rat

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**Background:** It seems that the sexual cycle is highly sensitive to environmental light-dark conditions; i.e., halted and irregular during short-day and long-day conditions, respectively. These effects are mediated by the pineal gland, which converts light-dark signals into a chemical signal (melatonin [Mel]).

**Objective:** The aims of this study were firstly, to evaluate the effects of long day (short night) conditions on the regularity of estrus cycle and the probable spatial working memory deficits and secondly, the curing

effects of oral administration of ML on the above parameters.

**Materials and Methods:** A total of 30 female Wistar rats (3-4 month, 220 ± 30 gr) were randomly divided into 6 groups (n = 5/each). Experimental groups were categorized as control, control + Mel, experimental 1, experimental 1 + Mel, experimental 2, and experimental 2 + Mel. Light conditions included standard light: dark (12 hr light/ dark cycle), short night (16 hr light/8 hr dark), and long night (20 hr light/4 hr dark) with free access to food and water was provided. They were exposed to different light conditions (light-dark cycles) for 6 wk. Mel was administered at a final concentration of 0.8 µg/mL dissolved in drinking water. Control groups received a placebo (ethanol). To check the regularity of the sexual cycle daily vaginal smear cytology was done. During the final week, elevated plus maze test was undertaken to assess the probable spatial working memory deficit.

**Results:** Female rats kept in long light conditions show severe irregularity in estrus cycles and Mel administration significantly reduced the number of irregular cycles (p = 0.0006). Exposure to night-time light and Mel treatment had no obvious effect on spatial working memory.

**Conclusion:** Based on the results obtained from the present study, it can be concluded that Mel administration may correct the disturbances of the sexual cycles via its strong anti-oxidant properties.

**Keywords:** Sexual cycle, Light pollution, Melatonin, Rat.

#### P-48

### The effects of chronic sleep deprivation combined with circadian rhythm disruption on fertilization rate and embryo development in BALB/c mice

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**Background:** Sleep is a crucial physiological process integral to daily functioning, and insufficient sleep is a significant risk factor for overall health. In contemporary society, various factors, including shift work and lifestyle changes, have adversely impacted sleep and circadian patterns. Given that sleep and circadian patterns physiologically regulate the synthesis, secretion, and metabolism of hormones essential for reproduction. Sleep deprivation has been identified as a contributing factor to infertility in both men and women; however, its simultaneous effect with the circadian rhythm disruption has not been investigated.

**Objective:** This study aimed to investigate the effects of chronic sleep deprivation and circadian rhythm

disruption on fertilization rate and embryo development in BALB/c mice.

**Materials and Methods:** In this experimental study, 40 female BALB/c mice (8 wk, 20-30 gr), were divided into 5 equal groups (n = 8/each): control group, circadian disruption, daily sleep deprivation (4 hr), night sleep deprivation (4 hr), and daily sleep deprivation with circadian disruption. The experimental conditions for the studied groups were implemented using a sleep deprivation device and a light cycle alteration schedule of 3.5 D: 3.5 L. After 4 wk, the mice were euthanized, and blood and oviduct samples were collected to evaluate cortisol hormone levels, cleavage, compact and blastocyst embryo formation rate.

**Results:** The findings of the investigation indicated that the mean weight difference of the mice before and after the intervention, and the fertilization rate, did not represent statistically significant differences compared to the control group. A notable increase in the mean cortisol hormone level was observed exclusively in the SD + CD group, alongside a significant reduction in the 2, 4, and 8-cell embryo formation rate within this group. Additionally, there was a considerable decline in the compact and blastocyst embryo formation rate across all intervention groups (p < 0.05).

**Conclusion:** Contrary to the notion that during the early stages of cleavage, only the combination of sleep deprivation and circadian disruption adversely impacts the cleavage embryo formation rate, in the late stages of cleavage, both sleep deprivation and circadian disruption, whether occurring separately or together, can detrimentally affect on the compact and blastocyst embryo formation rate.

**Keywords:** Embryos, Cortisol, Sleep deprivation, Circadian rhythm disruption.

#### P-49

### Comparative study of learning and memory capabilities during proestrous and diestrous stages of the sexual cycle in female Wistar rats

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**Background:** In females, during each sexual cycle, significant fluctuations occur in the production and secretion of ovarian steroid hormones (estrogen and progesterone in the follicular and secretory phases of the ovary, respectively). At the brain level, changes in the excitability of neural circuits caused by hormonal fluctuations manifest as changes in mood and emotional behaviors, etc.

**Objective:** The present study aims to study learning and memory capabilities during the diestrus (DIE) and proestrus (PRO) phases of the sexual cycle of rats.

**Materials and Methods:** In this experimental study 14 mature female Wistar rats (200-220 gr, 3-4 months) were randomly divided into PRO and DIE groups (n = 7/each). During the experimental period, all rats were

undertaken daily vaginal smear cytology and then entered into the behavioral tests: elevated plus maze (for anxiety assessment), open field (for seeking), shuttle box (for memory evaluation). At the end, the results of each test were statistically analyzed and compared between 2 groups.

**Results:** In the elevated plus maze test, the measured parameters were the percentage of entries into the open arm and the percentage of time spent in the open arms. The results showed that, in compare with PRO group, in DIE group the number of entries into the open arm and the time spent in the open arm were significantly reduced ( $p < 0.0001$ ). In the open field test the measured parameter was the time spent in the central boxes, in compare to peripheral boxes. The results showed that the seeking behavior was significantly higher in PRO group than the DIE group ( $p < 0.051$ ). In the shuttle box test, the measured parameter was the delay time for the entry into the dark chamber. The results showed that the latency to enter the dark compartment was significantly ( $p < 0.05$ ) longer the PRO group than DIE group.

**Conclusion:** It can be concluded that this difference in the above parameters are due to the effects of the fluctuations in sex hormones. It seems that, in compare to PRO group, rats in DIE group were more sensitive to stressfull conditions, less restless and more alert.

**Keywords:** Sexual cycle, Memory, Learning, Female rat.

## P-50

### Effects of theophylline on sperm parameters in asthenozoospermic men during cryopreservation

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**Background:** Asthenozoospermia (AZS), characterized by reduced or absent sperm motility in fresh ejaculates, is a leading cause of male infertility. Sperm freezing, an integral part of assisted reproductive technologies helps preserve male fertility but often compromises sperm quality. Theophylline, a xanthine derivative with antioxidant properties, functions as a phosphodiesterase inhibitor, enhancing intracellular cyclic adenosine monophosphate (cAMP) levels and cAMP-dependent processes like sperm motility. While theophylline has shown promise in improving viable sperm quality, its effect on sperm freezing in AZS individuals remains unclear.

**Objective:** This study aimed to evaluate the effect of theophylline on sperm parameters during the freezing-thawing process in AZS men.

**Materials and Methods:** In this experimental study, 30 semen samples from AZS men referred to the Roya

infertility treatment center at Qom University Jihad, Qom, Iran were collected into sterile containers through masturbation in 2023-2024. Each sample was divided into 3 groups: control (fresh semen), freeze (frozen with cryoprotectant only), and freeze + theophylline (frozen with cryoprotectant supplemented with 0.05 mM theophylline). Cryopreservation was performed using a human sperm-freezing medium and a rapid freezing method. Sperm motility, viability (assessed via eosin-nigrosin staining), and morphology (evaluated using the Diff-Quick Kit) were analyzed according to World Health Organization criteria.

**Results:** The freeze-thaw group showed a significant reduction in the mean percentages of sperm motility, viability, and normal morphology compared to the control group ( $p = 0.001$ ). In contrast, the freezing + theophylline group demonstrated a significant increase in sperm motility and viability ( $p = 0.001$ ) and an increase in normal morphology ( $p = 0.035$ ) compared to the freezing group.

**Conclusion:** This study demonstrated that supplementing the cryoprotectant medium with theophylline significantly improves sperm motility, viability, and morphology in AZS men, emphasizing its potential to reduce cryopreservation damage and enhance fertility outcomes in assisted reproductive technologies.

**Keywords:** Asthenozoospermic men, Cryopreservation, Theophylline, Sperm parameters.

## P-51

### Selenium enhances sperm quality in asthenozoospermic men during the freeze-thaw process

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**Background:** Sperm freezing is a widely used and essential technique for preserving and protecting fertility. However, despite its importance in assisted reproduction centers, the process can reduce sperm quality due to oxidative stress. Incorporating antioxidants into the freezing medium can help protect the structural and functional characteristics of sperm from freeze-thaw induced damage. Selenium, a trace biological element and potent antioxidant, plays a crucial role in sperm production and enhancing quality of sperm parameters.

**Objective:** In this study, the effect of selenium supplementation on sperm parameters in asthenozoospermic men during cryopreservation of semen samples was assessed.

**Materials and Methods:** In this experimental study, semen samples were collected from 30 asthenozoospermic men referred to the infertility treatment center (Roya) at Qom University Jihad, Qom, Iran between December 2023 and May 2024. Each sample was divided into 3 groups including control (fresh semen), freeze (frozen with sperm-freezing medium only), and freeze + selenium (frozen with sperm-freezing medium supplemented with 2 µg/mL selenium). Cryopreservation was performed using a human sperm-freezing medium and a rapid freezing method. Sperm motility, viability (assessed using eosin-nigrosin staining), and morphology (evaluated using diff-quick staining) were analyzed before and after freezing, following the World Health Organization guidelines.

**Results:** A significant reduction was found in the mean sperm motility, viability, and normal morphology in the freeze group compared to the control group ( $p = 0.001$ ). However, these parameters significantly increased in the freeze + selenium group when compared to the freeze group ( $p = 0.001$ ).

**Conclusion:** Our data indicate that supplementing the freezing medium with selenium reduces the adverse effects of the freeze-thaw process on sperm quality in asthenozoospermic men, presenting a promising approach to increasing fertility outcomes in assisted reproductive techniques.

**Keywords:** Asthenozoospermia, Cryopreservation, Selenium, Sperm parameters.

## P-52

### Investigating the effects of melatonin administration on change in ovarian morphometric indices caused by light pollution in Wistar rats

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**Background:** Light pollution is one of the most important factors disrupting biological rhythms in animals. In adult female mammals, the hypothalamus-pituitary-ovary axis is controlled by biological rhythms, and the effects of disturbances in this axis are manifested in its terminal part, namely, ovarian cycles. As the most important hormone regulating biological rhythms, melatonin plays an important role in reducing the effects of disruption in biological rhythms.

**Objective:** In this research, the effect of melatonin hormone administration on body weight, correction of ovarian cycle irregularity, ovarian structure, changes in catalase enzyme level and total serum antioxidant capacity, under the conditions of exposure to night light, have been investigated.

**Materials and Methods:** In this experimental study, 30 female Wistar rats ( $220 \pm 20$  gr, 3-4 months) with a regular sexual cycle were divided into 6 groups ( $n = 5$ /each) including: control, control+melatonin,

experiment 1, experiment 1+melatonin, experiment 2 and experiment 2+melatonin, respectively, under standard light conditions (12 hr light/dark cycle), short-term night light (16 hr light/8 hr dark) and long-term night light (20 hr light/4 hr dark) with free access to food and water. The groups treated with melatonin received melatonin as a solution in drinking water (At a dose of 0.8 µg/ml of water). During the test period, in order to evaluate the state of the sexual cycle, vaginal smear cytology was performed daily. At the end of the experimental period (day 42), under deep anesthesia, blood was taken from the rats first, and then the ovaries were removed and fixed in fixative solution. In the following, ovaries were used for histological investigations and blood serum was used to measure catalase enzyme level and total antioxidant capacity.

**Results:** The weight measurement of the rats showed that compared to the beginning of the test period, the weight of the rats decreased at the end of the test period and the administration of melatonin prevented weight loss in the rats. This effect is especially significant when comparing the control group with the control+melatonin group ( $p < 0.039$ ). The data obtained from the daily cytology of the vaginal smear indicate that the treatment with melatonin significantly prevents the occurrence of irregularity in sexual cycles caused by night light ( $p < 0.0006$ ). Examination of the structure of the ovary also showed that melatonin was able to prevent the decrease in the volume of the ovary ( $p < 0.0019$ ), decrease the number of corpora lutea ( $p < 0.0252$ ), and decrease the ratio of the area of the corpora lutea to the cut area of the ovary ( $p < 0.0076$ ). Treatment with melatonin also caused a significant decrease in the serum level of catalase enzyme ( $p < 0.0002$ ). At the same time, administration of melatonin did not significantly change the total serum antioxidant capacity.

**Conclusion:** Based on the obtained results, it can be concluded that melatonin hormone administration; 1) it prevents cyclic disorders in the hypothalamus hypophysis-ovarian axis caused by night light. 2) as a powerful natural antioxidant, it prevents tissue damage caused by oxidative stress, which is likely to occur during exposure to night light.

**Keywords:** Light pollution, Melatonin, Ovary, Oxidative stress.

## P-53

### Melatonin mitigates oxidative stress in asthenozoospermic men during freeze-thawing process

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**Background:** Asthenozoospermia, characterized by reduced or absent sperm motility, is a major cause of

male infertility. Sperm cryopreservation, a vital technique in assisted reproductive technologies, induces cryoinjury, compromising sperm structure and function through oxidative stress, DNA damage, and apoptosis. Excessive reactive oxygen species (ROS) production during freeze-thaw cycles exacerbates oxidative stress, particularly in asthenozoospermic men, impairing semen quality and fertilization potential. Melatonin, a potent antioxidant, may mitigate these effects.

**Objective:** This study aimed to evaluate the effects of melatonin, on sperm biochemical parameters and intracellular ROS levels in cryopreserved samples from asthenozoospermic men.

**Materials and Methods:** This experimental in vitro study involved semen samples from 30 asthenozoospermic men, collected at the Amir-AL-Momenin Infertility Center, Arak, Iran from November 2023 to Jun 2024. Samples were divided into 3 groups: control (fresh), freeze, and freeze + melatonin (treated with cryoprotectant + 1 mM melatonin). Samples in the freeze groups were cryopreserved using a human sperm freezing medium and rapid freezing protocol. Levels of malondialdehyde (MDA), total antioxidant capacity, and antioxidant enzymes (catalase, glutathione, superoxide dismutase) were quantified using enzyme-linked immunosorbent assay. Intracellular ROS levels were measured using the DCFH-DA fluorescent probe via a Partec Pas flow cytometer with a 488 nm argon laser.

**Results:** The mean levels of sperm antioxidant enzymes and total antioxidant capacity in the freezing group were significantly reduced compared to the control group ( $p < 0.001$ ). In contrast, the mean levels of sperm MDA and ROS in the freezing group showed a significant increase ( $p < 0.001$ ). A significant increase in the sperm antioxidant enzymes and TAC levels ( $p < 0.001$ ), along with a significant decrease in MDA ( $p < 0.001$ ) and ROS ( $p = 0.005$ ) levels were seen in the freeze + melatonin group compared to the freezing group.

**Conclusion:** In conclusion, incorporating melatonin into the freezing medium significantly reduces the adverse effects of cryopreservation on the sperm of asthenozoospermic men by mitigating oxidative stress and lowering ROS levels. This protective role of melatonin highlights its potential as an effective antioxidant in improving the quality and functionality of cryopreserved sperm, ultimately contributing to better outcomes in assisted reproductive technology.

**Keywords:** Melatonin, Cryopreservation, Asthenozoospermia, Reactive oxygen species, ART.

## P-54

### Effects of in vitro and in vivo oocyte aging on fertilization and embryo development in BALB/c mice

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**Background:** Following ovulation, oocytes become arrested in the metaphase of meiosis II. Without fertilization, they experience a gradual decline in quality over time, known as post-ovulatory aging (POA). Although POA affects the outcomes of assisted reproductive technology, its underlying mechanisms are largely unknown.

**Objective:** Given the critical importance of POA's effects on oocyte quality, fertilization capacity, and subsequent embryonic development, the current experimental study evaluated the impact of 24 hr in vitro and in vivo oocyte aging on fertilization rate and embryo development.

**Materials and Methods:** In this experimental study, 24 female BALB/c mice (20-25 gr, 8 wk) were randomly divided into 3 groups ( $n = 8$ /each) of a control group, an in vivo aging group, and an in vitro aging group. Superovulation was induced by an intraperitoneal injection of 5 IU of human menopausal gonadotropin, followed 48 hr later by an intraperitoneal injection of 10 IU of human chorionic gonadotropin. In the control and in vitro aging groups, after 16 hr, the mice were sacrificed to obtain oocytes. In the in vitro aging group, the collected oocytes were transferred into 100- $\mu$ l drops of human tubal fluid without HEPES medium and cultured under mineral oil at 37°C for 24 hr in a humidified atmosphere containing 5% CO<sub>2</sub>. For the in vivo aging group, after 24 hr, the mice were sacrificed to obtain oocytes that had undergone in vivo aging process. In vitro fertilization was done after oocyte collection in control group and in vivo aging groups, and after 24 hr oocyte incubation in in vitro aging group. Fertilization, cleavage, and blastocyst formation rates were evaluated in different groups.

**Results:** There was no statistically significant difference in the fertilization rate in in vivo aging ( $p > 0.999$ ) and in vitro aging ( $p = 0.226$ ) groups compared to the control group. The formation rate of 2 ( $p = 0.046$ ), 4 ( $p = 0.034$ ), and 8-cell ( $p = 0.003$ ) embryos was significantly reduced only in the in vitro aging group. In vivo group showed a significant decrease in compact ( $p = 0.001$ ) and blastocyst ( $p = 0.002$ ) embryo formation rates compared to the control group. The decrease in in vitro group was also observed in both compact ( $p < 0.001$ ) and blastocyst ( $p = 0.001$ ) embryo formation rates.

**Conclusion:** While early cleavage stages are primarily affected by in vitro oocyte aging, late cleavage stages can be negatively impacted in both intervention groups. The negative effect of in vitro oocyte aging compact and blastocyst embryo formation rates was higher than that of in vivo oocyte aging.

**Keywords:** Oocyte, Aging, In vitro, In vivo.

## P-55

### Chemical pregnancy in fresh and cryopreserved cycles, using day 3 (cleavage stage), and day 4 embryos (morula stage)

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**Background:** With the advancement in the embryo culture media which supports nutrient requirements of embryos up to 5-6 days, there's a chance to select more viable embryos, which are more likely to result in pregnancy, compared to earlier stages. Also, there is a controversy regarding the freeze embryo transfer, compared to the fresh type.

**Objective:** To compare the chemical pregnancy rates between fresh embryo transfer, and frozen embryo transfer, on day-3 (cleavage), and day-4 (morula) of development.

**Materials and Methods:** In this retrospective study, data of 242 fresh, and 758 frozen embryo transfer cycles was obtained, in one infertility center in Isfahan, Iran. The study's groups were assigned based on the day of fresh or freezing embryo transfer (day-3, or day-4 embryos) and the embryo grading. Chemical pregnancy was the main outcome measurement (Implantation rates).

**Results:** The chemical pregnancy rate was higher in the good quality freeze embryo day-3 and transfer on day-4 group (40.1%). This rate was near the results of transferring the good quality freeze embryo on day-4 (39.2 %). There was no significant difference in the chemical pregnancy rate, related to the number of transferred embryos ( $p = 0.55$ ).

**Conclusion:** The higher PRs when the embryos were transferred on day-4, provided further support for the morula stage embryo transfer, possibly because of better synchrony with the endometrium. It is concluded that morula/compact embryos are good candidates for embryo transfer, which simultaneously reduces the number of transferred embryos.

**Keywords:** Embryo development, Endometrial receptivity, Embryo transfer, Synchrony, Freeze embryo, Fresh embryo.

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## **P-56** **S100 protein family and embryo implantation**

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It is well known that embryo implantation is a critical process in which embryo should be able to reach and attach to endometrium. Until now, various types of factors are involved in the regulation of this process. S100 proteins are calcium-binding proteins, which have vital roles in embryo implantation and have been considered as possible candidate markers for endometrial receptivity. However, studies regarding mode of actions of these proteins are scarce and more mechanistic insights are needed to clarify exact roles of each one of the S100 protein family. Understanding of function of these proteins in different compartments, stages, and phases of endometrium, could pave the way for conducting studies regarding the therapeutic significance of these proteins in some disorders such as recurrent implantation failure. In this review, we outlined roles and possible underlying mechanisms of S100 protein family in embryo implantation. S100 family proteins exert various types of functions during critical process of fertility specially embryo implantation. Almost all member of this family regulates endometrial receptivity via calcium-dependent mechanisms. However, more studies are needed to unravel exact roles of these proteins. Fortunately, omic studies introduced some member of this family as a potential endometrial receptivity biomarker, and it could be a hope for targeted therapy of disorders such as RIF and endometriosis base on the regulation of these proteins. For achieve this goal, it is essential to find novel mode of actions of S100 family proteins to design new and effective molecular targeted therapy for fertility problems. S100 family proteins exert various types of functions during critical process of fertility specially embryo implantation.

**Keywords:** Embryo implantation, Recurrent implantation failure, S100 proteins.

## **P-57** **Umbilical cord blood as valuable resource for applicable stem cells: Aggregated procedures and biomolecular contents in a systematic review**

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**Background:** Umbilical cord blood have a reproductive and high proliferation capacity compared with bone

marrow and peripheral blood cells. Main components of umbilical cord blood are including the hematopoietic, mesenchymal, pseudo-embryonic, unlimited somatic stem cells and, endothelial cells, lymphocytes, natural killer cells, dendritic cells and erythrocytes.

**Objective:** The aim of this study was identifying the key biomolecules and application of umbilical cord blood as valuable source for applicable stem cells.

**Materials and Methods:** In this systematic review study 20 of 200 papers from the PubMed, Google scholar, Cochran and Scopus entered to the study by systematic review.

**Results:** Umbilical cord blood was aggregated by tow procedures: intrauterine method in which the stem cells of umbilical cord blood received from placenta or cord blood vessels after childbirth and before than placental abruption and the second method is extra uterine that umbilical cord blood stem cells aggregated after placental abruption. Special bag with barcode chooses after clamping and cutting the umbilical cord. The needle attached to the bag is inserted into the umbilical vein under sterile conditions. The bag is placed at a lower level so that the blood inside the umbilical cord vein is quickly and completely transferred into the sterile collection bag contained with anticoagulant.

**Conclusion:** The presence of different cell populations in umbilical cord blood alongside hematopoietic stem cells makes it possible to use this resource in immunotherapy, tissue engineering and regenerative medicine. Therefore, isolation and expansion of different cell subtypes from umbilical cord blood and their use in the treatment of various diseases, especially in close relatives, have been considered.

**Keywords:** Umbilical cord blood, Stem cell, Transplantation.

## P-58

### Fertility preservation in the digital age: Opportunities and challenges of online resources for individuals with cancer

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Cancer ranks as the leading cause of mortality worldwide. Even though the survival rate has increased over the past years due to advancements in treatment approaches, the occurrence of health issues, particularly infertility, has been raised, which can stem from the cancer itself and/or the treatment process. However, fertility preservation services are either under-prescribed or under-utilized. Studies suggest that low levels of awareness and knowledge are a critical barrier to fertility preservation decision-making in cancer survivors. Hence, researchers have turned to internet-based approaches as a solution, which are now recognized as an accessible approach to obtaining

information among most populations. This study aimed to review the quality and accessibility of online resources for fertility preservation among cancer survivors, identifying gaps in information, readability, and inclusivity to enhance people's knowledge and support informed decision-making regarding oncofertility care. To this end, literature review was conducted using digital databases such as PubMed, Science Direct, and Scopus. Studies revealed that a significant portion of websites related to the provision of fertility preservation information is from general or pediatric hospitals and cancer-related non-profits. Results indicated that many sites scored poorly in quality. According to the reports of studies, 83.8% of the websites provided limited information on uncertainties related to treatments, and 97% lacked adequate references. Furthermore, the majority failed to demonstrate a balanced perspective. The average reading level was 12<sup>th</sup> grade, indicating higher complexity in language. In terms of features, studies revealed that while most included basic facts on fertility preservation, interactive elements like survivor stories were rarely present. Regarding gender representation, more than half of the sites catered to both male and female audiences, covering various fertility preservation methods. However, socioemotional discussions surrounding costs and emotional impacts were notably sparse, particularly for transgender considerations. Online resources for people with cancer have potential but currently lack quality, thoroughness, readability, appeal, and inclusivity in fertility preservation websites. Clinicians and researchers have a valuable opportunity to collaborate with adolescents and young adults to develop effective educational websites that improve the knowledge of people with cancer and encourage shared decision-making about oncofertility care.

**Keywords:** Digital health, Fertility preservation, Patient education, Shared decision-making.

## P-59

### Comparison of moderate and high doses of fennel seed on serum antioxidant level and folliculogenesis in F1 female mice offspring

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**Background:** In Iranian lore, *Foeniculum vulgare* (fennel) is traditionally used to treat of various disease because of its antioxidant and phytoestrogen compounds.

**Objective:** This study was carried out to evaluate the total antioxidant capacity (TAC) and folliculogenesis in offspring exposed to moderate and high doses of fennel seed in the prenatal period, breastfeeding until 56 postnatal days (PND 56).

**Materials and Methods:** 24 adults female NMARI mice (25-30 gr 6-8 wk) were randomly divided into 3 groups (n = 8): control (CTL), and seed-treated groups

that received 500 and 1000 mg/kg/day fennel seed (FS-500, and FS-1000). The treatments started from pregnancy day 1 and continued until PND 56. Their body and ovary weight and diameter were measured. The ovary was removed, fixed and 5 $\mu$  sections were stained using the H&E method to assess folliculogenesis. The serum level of TAC was measured using ELISA kit.

**Results:** The results showed that body and ovary weights significantly increased in the FS-treated groups in comparison with the CTL group (FS-500;  $p < 0.05$ , FS-1000;  $p < 0.01$ ). A significant increase of the mean number of primordial, primary, pre-antral, pre-ovulatory follicles was observed in both doses of the FS -treated animals in comparison with the CTL group ( $p < 0.05$ ). Also, corpus luteum count was raised in both FS-treated mice, although it reached to a significant difference in the FS-1000 groups when compared to the CTL group ( $p < 0.01$ ). Moreover, there was a significant higher level of TAC in the FS-500 group compared to the CTL group ( $p < 0.001$ ).

**Conclusion:** The results of the present study showed that exposure to fennel seed could improve the folliculogenesis in the ovary of first-generation mice. This may be a result of the elevated antioxidant capacity level.

**Keywords:** *Foeniculum vulgare*, Folliculogenesis, Ovary, First generation, Mice.

## P-60

### Ferulic acid enhances the post-thaw quality of cryopreserved human sperm while mitigating oxidative stress

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**Background:** Human sperm cryopreservation is widely recognized as a standard clinical method for long-term preservation. However, the process often leads to cryoinjury, primarily driven by oxidative stress, which negatively affects semen quality. Research suggests that incorporating antioxidants can help safeguard sperm during freezing and thawing.

**Objective:** This study evaluated the impact of 2 ferulic acid concentrations (25 and 50  $\mu$ g/ml) on oxidative status and the quality of cryopreserved sperm.

**Materials and Methods:** This experimental research involved 30 normospermic donors who provided semen samples via masturbation and referred to Hazrat Zeinab Educational Medical Center, Shiraz, Iran. We used normospermic samples since our goal was the evaluation of ferulic acid as an antioxidant on sperm parameters after cryopreservation. Each sample was divided into 4 groups: fresh sperm without cryopreservation, cryopreserved sperm without supplements, and cryopreserved sperm supplemented with either 25  $\mu$ g/ml or 50  $\mu$ g/ml of ferulic acid. Various parameters, including semen quality, sperm DNA integrity, chromatin dispersion, and oxidative stress markers, were assessed.

**Results:** Results indicated that the beneficial effects of 50  $\mu$ g/ml ferulic acid on sperm parameters were more significant compared with 25  $\mu$ g/ml. 50  $\mu$ g/ml of ferulic acid significantly enhanced sperm morphology, motility, and viability. Additionally, this dosage effectively restored DNA and membrane integrity compromised by the freeze-thaw cycle ( $p \leq 0.001$ ). Ferulic acid also demonstrated strong antioxidant activity by reducing total oxidant status, increasing total antioxidant capacity, and lowering malondialdehyde level as a marker of lipid peroxidation ( $p \leq 0.001$ ).

**Conclusion:** These findings suggest that supplementing cryopreservation media with 50  $\mu$ g/ml ferulic acid is a promising approach to mitigate oxidative stress-induced cryoinjury, though further research is recommended.

**Keywords:** *Ferulic acid*, Spermatozoa, Cryopreservation, Oxidative stress.

## P-61

### How do swim-up and density gradient centrifugation methods affect sperm biological parameters, DNA fragmentation, and separation of spermatozoa containing X and Y chromosomes?

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**Background:** According to the National Infertility Prevalence Survey, the infertility rate in Iran ranges from 18.3-22.5% (20.3%) with a 95% confidence interval.

**Objective:** We aimed to evaluate the effect of conventional sperm processing methods (swim-up, density gradient centrifugation [DGC], and sperm wash) on biological parameters (total count, motility, progressive and non-progressive motility, and morphology), DNA fragmentation, and the expression of sex-determining Region Y (*SRY*) and Transducin  $\beta$ -

Like protein 1, X-linked (*TBLIX*) genes in processed spermatozoa.

**Materials and Methods:** Seven semen samples from Holstein cattle at Jahid Livestock Inputs Co. were transferred to the laboratory (20 min/ 25°C/ in the dark). 7 semen samples from Holstein cattle at Jahid Livestock Inputs Co. were transferred to the laboratory (20 min/ 25°C/ in the dark). The control group was washed with Ham's F10 (1:1) and centrifuged at 700x g for 7 min. DGC involved mixing semen with 80% and 40% SpermGrad and centrifuging at 500x g for 5 min. Sperm biological parameters (total count, motility, progressive and non-progressive motility, and morphology) were evaluated using a computer-assisted semen analyser. *SRY* (spermatozoa containing X chromosome) and *TBLIX* genes (spermatozoa containing Y chromosome) expressions were detected using reverse transcription-polymerase chain reaction. DNA fragmentation and chromatin integrity in each group were detected using papanicolaou and aniline blue staining.

**Results:** The total sperm counts were significantly higher in the control ( $26.3 \pm 3.4 \times 10^6$ ) and DGC groups ( $24.2 \pm 2.8 \times 10^6$ ) than in the swim-up method ( $11.99 \pm 1.2 \times 10^6$ ). The highest and lowest total motility were detected in the swim-up and control groups ( $74.51 \pm 5.6\%$  and  $50.76 \pm 4.9\%$ , respectively) ( $p = 0.023$ ). The swim-up method also showed better progressive motility ( $57.96 \pm 5.8\%$ ) than the control ( $38.34 \pm 2.7\%$ ) ( $p = 0.042$ ). Non-progressive and immotile sperm were lower in the swim-up method ( $10.59 \pm 3.5\%$  and  $22.27 \pm 2.7\%$ ) versus the control ( $16.54\%$  and  $48.91\%$ ). DGC and swim-up processing improved sperm chromosomal structure and integrity ( $p = 0.028$ ). Sperm chromatin integrity in the control group was  $28 \pm 1.22\%$ , significantly lower than in the other groups ( $p = 0.028$ ). The control and swim-up methods showed the highest and lowest levels of DNA fragmentation and chromatin integrity ( $p = 0.034$ ). Sperm processing methods significantly influenced the separation of X and Y chromosome-bearing sperm, with DGC having the most X chromosomes and swim-up the most Y ( $p = 0.029$ ). *SRY* gene expression was highest in the swim-up ( $76.58\%$ ) and lowest in the control ( $53.52\%$ ) ( $p = 0.035$ ).

**Conclusion:** Sperm processing methods affect the biological parameters, chromatin integrity, DNA fragmentation, and the percentage of sperm containing X and Y chromosomes.

**Keywords:** *SRY*, *TBLIX*, Sperm, Swim-up, DGC, Fragmentation.

## P-62

**The effect of silymarin (*silybum marianum*) extracts on the parameters, DNA integrity, and free radical levels of normal male sperm after freezing by the rapid freezing method**

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**Background:** Cryopreservation of human sperm by rapid freezing significantly reduces sperm motility, morphology, and DNA integrity. Silymarin seed extract contains a high concentration of an active compound which has shown an antioxidant effect by eliminating reactive oxygen species and inhibiting lipid peroxidation.

**Objective:** This project aimed to check whether silymarin extract as a strong antioxidant has a protective role for sperm against the freezing process for sperm.

**Materials and Methods:** In this experimental study, we used 20 normospermia samples who referred to Yazd Reproductive Science Institute, Yazd, Iran from January to Jun 2024. The samples are divided into 4 groups. The control groups 1 and 2 (before and after freezing) and the experimental groups sperm freezing media supplemented by doses of 500 and 100 mg/ml for silymarin extract. Sperm parameters were assessed in terms of motility, viability, morphology, protamine deficiency, and DNA fragmentation. A malondialdehyde kit was used to evaluate the reactive oxygen species level.

**Results:** The use of silymarin extract in a dose of 100 mg/ml significantly increased the percentage of progressive sperm, as well as viability ( $p = 0.003$ ), and also decreased DNA fragmentation ( $p = 0.021$ ) compared to the freezing group without silymarin extract. The use of silymarin in 2 doses of 100 and 500 mg/ml did not significantly change the percentage of sperm with normal morphology. The addition of silymarin at a dose of 100 and 500 has significantly decreased the amount of sperm malondialdehyde compared to the freezing group ( $p = 0.002$  and  $p = 0.01$ , respectively). Finally, the addition of 100 and 500 silymarin doses significantly reduced sperm protamine deficiency ( $p < 0.001$ ).

**Conclusion:** Sperm freezing can reduce the fertility potential. Therefore, to overcome this problem and improve the results of assisted reproductive methods, it is suggested to enrich sperm freezing with the use of biological and protective substances such as silymarin extract. Data showed the use of silymarin extract especially in a dose of 100 mg/ml dramatically improved sperm parameters and DNA conditions after the freezing procedure. Using these materials in the sperm freezing process, which is still in the research phase, can promise less damage to frozen and thawed sperm.

**Keywords:** Silymarin, DNA integrity, Freezing, Spermatozoa.

## P-63

**The effect of green tea extract on the parameters, DNA integrity, and the amount of free radicals of normal sperm after freezing by rapid freezing method**

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**Background:** During the freezing-thawing process of sperm, physical and chemical stresses are created in the sperm membrane and cause a decrease in the quality, viability, mobility, and fertility potential of sperm. These damages are caused by excessive production of free radicals, especially reactive oxygen species. Tea extract is a plant that has strong antioxidant properties and we can use its extract as a freeze protectant.

**Objective:** This study aimed to check the effects of green tea extract on sperm parameters, reactive oxygen species level, and DNA status.

**Materials and Methods:** In this experimental study, we used 20 normozoospermia samples who referred to Yazd Reproductive Science Institute, Yazd, Iran between Jan-May 2024. The samples are divided into 4 groups: the control, freezing, and experimental groups, including freezing media supplemented by doses of 4.0 µg/ml and 0.4 µg/ml of green tea extract (prepared according to the previous studies). Sperm freezing was done by the rapid freezing method. Sperm parameters were evaluated regarding motility, viability, morphology, protamine deficiency and DNA fragmentation. A malondialdehyde kit was used to evaluate the ROS level.

**Results:** The results showed that sperm parameters decreased and also DNA fragmentation and protamine deficiency increased significantly after the freezing process compared to the control group ( $p < 0.001$ ). The use of doses of 0.4 µg/ml of green tea extract significantly increased the percentage of progressive sperm ( $p = 0.021$ ), as well as viability ( $p = 0.016$ ), and also decreased DNA fragmentation ( $p = 0.021$ ), and protamine deficiency ( $p = 0.024$ ) compared to the freezing group without green tea extract. The use of green tea extract in doses of 4.0 mg/ml did not significantly change the percentage of none of sperm parameters.

**Conclusion:** The use of anti-freezing protectants can reduce the damage caused by the freezing procedure. Our data showed that the doses of 0.4 µg/ml green tea significantly improved the sperm parameters and DNA condition.

**Keywords:** *Green tea, DNA integrity, Freezing, Sperm motility.*

#### P-64

### **Extracellular vesicles as a potential tool in improving in vitro maturation rate**

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Extensive communication between follicular somatic cells and the oocytes is essential for ovarian follicle growth and maturation. Recently, it has been reported that extracellular vesicles (EVs), including exosomes, have been identified in ovarian follicular fluid, which contains miRNA and proteins, and is involved in intercellular communication. EVs affect numerous cellular functions by regulating various signaling pathways and they have a similar therapeutic effect to their cells of origin. Hence, there is a probability that follicular fluid contains a remarkable source of bioactive factors such as EVs that can be used as supplements to improve oocyte growth during in vitro maturation (IVM). In this review, PubMed, Scopus, and Google Scholar databases were searched for the studies published from 2015-2024 with Keywords "Extracellular vesicle", "Exosomes", and "IVM". The search and data extraction were conducted by 2 authors. Based on these studies, the effect of supplements such as EVs on improving IVM has been investigated. Evidence suggests that exosomes obtained from mesenchymal stem cells may help restore ovarian function in mice with ovarian failure. Moreover, laboratory studies have shown that these exosomes inhibit cell death in Chinese hamster ovary cells. Based on this evidence, EVs have been identified as a novel source of biomarkers and potential reproductive medicine therapeutics, especially for assisted reproductive technology. There are still many technical and scientific hurdles that need to be overcome before EVs can be used in clinical assisted reproductive technology diagnostic and therapeutic applications. Also, clinically stable and robust EV isolation procedures have yet to be developed. Findings suggest that adding EVs to the IVM culture medium creates an optimal environment that can improve oocyte maturation rates. Hence, the addition of EVs into the IVM medium may ameliorate the cytoplasmic maturation of human immature oocytes and subsequent clinical outcomes.

**Keywords:** *Exosomes, Extracellular vesicles, IVM.*

#### P-65

### **An online intervention relationship enhancement couple therapy program versus systematic desensitization on sexual performance anxiety in women undergoing infertility treatment with timed intercourse: An RCT**

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**Background:** Sexual performance anxiety is common among infertility treatments. Psychological interventions are options for couples who do not tolerate psychiatric medications or like psychotherapy with pharmacotherapy.

**Objective:** This study aimed to compare the effectiveness of relationship enhancement couple therapy program (RECT) program versus systematic desensitization (SD) on sexual anxiety.

**Materials and Methods:** A randomized, parallel-group clinical trial was conducted on 68 (34 in each group) women with infertility and sexual anxiety who referred to the Hazrat Zahra Infertility Treatment Clinic in Shahrekord, Iran, in January 2022. Inclusion criteria included Iranian language, having a medical record of primary infertility in the mentioned center, having sexual intercourse anxiety based on the interview and sexual anxiety scale questionnaire, having a smartphone and internet literacy, having a sexual relationship with her husband, willingness to participate in the study and satisfaction of the husband for the woman's participation in the study. Exclusion criteria included pregnancy, ovarian hyper stimulation syndrome, having neurological and psychiatric diseases based on their medical report and women's self-report, taking psychiatric drugs based on infertility records, misfortune events such as the death of near relatives in the last 2 months, family quarrels in the last month, living in the location with no access to the internet, and participation in psychological counseling sessions or similar studies in the last 6 months. Women were randomly (simple randomization) assigned to one of the 2 groups of 34 people based on random allocation software. They were randomly (simple randomization) allocated to 8 weekly online interventions RECT or SD in the skyroom. The main outcome variables; sexual performance and anxiety, were assessed via Davis's questionnaire at baseline, completion of the intervention (week 8) and follow-up (week 12). The secondary outcome variable spouse's satisfaction was assessed (weeks 8 and 12, via visual analog scale). Data from 29 women were analyzed in each group (withdrawal of 10 women, n = 5/each).

**Results:** Intra-group comparison RECT and SD showed a significant decrease of main outcome variable sexual anxiety baseline compared to week 12 ( $p = 0.001$ ,  $0.001$  for RECT and SD, respectively). No significant difference was observed between the 2 groups in sexual anxiety in baseline compared to week 12 (RECT vs. SD,  $p = 0.149$ ). Time-group interaction of spouse's satisfaction showed a significant difference between RECT and SD ( $p = 0.010$ ).

**Conclusion:** Both RECT and SD were almost equally effective in decreasing sexual anxiety. RECT (couple relationship-centred) by empowering interpersonal ten

skills and SD (personal-oriented), by learning step-by-step how fight-or-flight response is replaced by relaxation response, helping women perform more adaptive behaviors regarding sexual anxiety. RECT increased spouse satisfaction significantly compared to SD, indicated males need to participate in positive relationship changes. The results may be applied by therapists and health policy makers.

**Keywords:** Psychological interventions sexual health, Infertility, Women, RCT.

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## P-66

### Effect of online problem-solving counseling on the sexual anxiety and intimacy of women with recurrent pregnancy loss: An RCT

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**Background:** Recurrent pregnancy loss (RPL) creates complex reproductive conditions among women. Problem-solving therapy is one of the sexual health approaches.

**Objective:** This study was designed to investigate the effect of online problem-solving counseling on the sexual anxiety and intimacy of women with RPL.

**Materials and Methods:** A randomized clinical trial was conducted at the Abortion Research Center of Yazd Reproductive Sciences Institute, Yazd, Iran between March and August 2023. A total of 70 women with RPL were randomly assigned into 2 groups, of intervention and control, using random allocation software ( $n = 35$ /each). The intervention group received 8 sessions of sexual counseling on online problem solving (the content of the sessions includes 1) getting the therapist and group members to know each other and giving explanations about the terms habitus abortion and problem solving approach, 2) talking about the client's experiences about habitus abortion, teaching logical problem solving and defining the problem in terms of clear and objective to be practical to solve the problem, 3) setting realistic goals to solve the problem, identifying the symptoms under the control of the individual, using practical exercises by the members to find negative thoughts related to their problem and illness, 4) implementation of the strategy brainstorming, teaching emotion-oriented and problem-oriented coping methods, 5) learning functional ABCs, 6) teaching effective real-life problem solving by combining emotions and logical thinking, teaching how to evaluate and choose a solution and identify its positive and negative points, 7) choosing the best solution by

members, 8) reviewing the next steps of progress, encouraging all progress). The control group received an online educational pamphlet. Sexual anxiety, and sexual intimacy were collected using questionnaires based on sexual anxiety and intimacy. The online questionnaires were completed before, after, and 1 month after the study.

**Results:** A total of 70 participants were included in the final analysis. The mean score of sexual anxiety in the 8<sup>th</sup> and 12<sup>th</sup> wk was significantly less in the online group than in control group ( $p < 0.001$ ). The mean score of sexual intimacy in the 8<sup>th</sup> and 12<sup>th</sup> wk was significantly higher in the online group than in the control group ( $p < 0.001$ ).

**Conclusion:** Problem-solving-based sexual health counseling programs may improve sexual anxiety and intimacy in women with RPL. It is recommended to use a sexual health counseling method in RPL centers when considering the effectiveness of this type of training.

**Keywords:** Anxiety, Problem-solving, Sexuality, Counseling, Habitual miscarriage.

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## P-67

### Investigating the effect of various psychological interventions on anxiety, depression, and stress in women who experienced repeated miscarriage: A review study

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Recurrent miscarriage is a serious challenge in women's reproductive health, defined as the loss of 2 or more consecutive pregnancies. This issue affects not only physical health but also the mental health of women, potentially leading to a wide range of psychological disorders. Women with a history of recurrent miscarriage often face psychological problems such as anxiety, depression, and stress, which can significantly negatively impact their quality of life and well-being. In recent years, psychological interventions have emerged as practical approaches to alleviate these disorders. The present study aims to determine the effectiveness of various psychological interventions on anxiety, depression, and stress in women with recurrent miscarriage experiences by reviewing existing studies and scientific evidence. This study was conducted as a narrative review. A search was conducted for studies published between 2010 and 2024 across PubMed, Science Direct, Google Scholar, Magiran, SID, and UpToDate. The search utilized keywords such as

"Interventions" "Psychological Interventions" "Cognitive Behavioral Therapy (CBT)", "Mindfulness-Based Stress Reduction (MBSR)" "Recurrent Miscarriage" "Anxiety" "Depression" and "Stress", along with their Persian equivalents. Included articles were published in English or Persian and evaluated the effectiveness of psychological interventions in reducing anxiety, depression, and stress in women with recurrent miscarriages. Various quantitative and qualitative studies relevant to the objective were selected and reviewed using a researcher-developed checklist. In total, 132 studies were reviewed, leading to the selection of 34 studies, which include randomized controlled trials and cohort studies focused on psychological interventions such as CBT, MBSR, supportive counseling, emotion-focused therapy, and hope therapy. The findings indicate that MBSR and CBT are recognized as the most effective methods for reducing anxiety and depression. These interventions are typically delivered in 8-12 sessions, and significant reductions in the average scores of various psychological indicators, including stress, anxiety, and depression, have been observed in women with recurrent miscarriages after completing the sessions. Supportive counseling has also led to a decrease in levels of depression and stress in limited studies; however, it did not significantly impact anxiety levels. Emotion-focused therapy and hope therapy have generally positively influenced the improvement of psychological status and the enhancement of resilience in women with recurrent miscarriages. Psychological interventions play a crucial role in enhancing the mental health of women with recurrent miscarriages. Counseling approaches such as MBSR and CBT have been identified as the most effective methods for reducing anxiety and depression. These findings emphasize the importance of incorporating psychological interventions into the routine care of women with recurrent miscarriages. Therefore, attending to psychological aspects alongside clinical care can help improve the overall condition of women experiencing recurrent miscarriages. However, it is important to recognize that the effectiveness of psychological interventions can be affected by factors such as individual characteristics, social support, and the quality of implementation. Thus, future research should focus on a more detailed examination of these factors.

**Keywords:** Psychological interventions, Recurrent miscarriage, Anxiety, Depression, Stress.

## P-68

### Psychological aspects of infertility: A systematic review

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**Background:** Infertility can impose a substantial psychological burden on individuals and couples. The journey often involves a complex mix of emotions, including feelings of sadness, frustration, and isolation.

**Objective:** This systematic review aimed to explore the diverse psychological dimensions related to infertility.

**Materials and Methods:** A comprehensive search was conducted in Medline, Web of Science Core Collection (indexes = SCI-EXPANDED, SSCI, A&HCI Timespan), and Scopus databases with the keywords; "infertility OR fertility", "emotional distress OR mental distress OR psychological distress OR depression OR stress OR mental disorders OR anxiety" and a combination of these words to find quality articles published from 2000-2024. Observational studies in English related to the purpose of the study were included in the analysis, and review studies, case reports, letters to editors, comments, and reports were excluded.

**Results:** In the initial search, 1075 articles were obtained. After screening and eligibility assessment, 38 studies were reviewed. The literature highlights several psychological aspects associated with infertility, including emotional distress, identity and self-esteem challenges, relationship strain, and social isolation. Emotional distress encompasses a range of feelings, such as grief and loss due to unmet reproductive goals, as well as heightened anxiety and depression stemming from the constant stress of fertility struggles. The journey through infertility can disrupt an individual's sense of identity, especially when societal norms link personal worth to fertility and parenthood. Additionally, relationship strain frequently develops as partners may deal with the emotional turmoil differently, leading to misunderstandings and conflicts. Lastly, many individuals feel unable to share their struggles with friends and family, leading to a sense of social isolation.

**Conclusion:** The psychological aspects of infertility are complex and multifaceted. It's essential for those experiencing infertility to seek support, whether through professional counseling or community resources, to navigate the emotional challenges they face.

**Keywords:** Psychological distress, Infertility, Systematic review.

## P-69

### The role of sexual health literacy in predicting women's sexual function and marital intimacy in working women of Yazd city

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**Background:** Sexual health is an important part of individual well-being, yet in many societies, it is often ignored due to sociocultural factors and the stigma embarrassment in talking about sexual issues. Sexual

health literacy consists of various domains such as sexual and gender development, puberty, pregnancy, contraception, unwanted pregnancy, sexually transmitted infections, communication about sexual preferences and coercion.

**Objective:** This study aims to investigate the role of sexual health literacy in predicting women's sexual performance and marital intimacy in married working women in Yazd, Iran city in 2022-2023.

**Materials and Methods:** In this cross-sectional study, 150 working married women who had no known psychiatric or physical disorders that referred to comprehensive health centers between October 2022 and March 2023 were selected by available sampling in Yazd, Iran. Participants completed the Walker and Thompson marital intimacy questionnaire, the sexual performance, and the adult sexual health literacy questionnaires.

**Results:** There was a significant relationship between sexual Health literacy, sexual performance, and marital intimacy among married working women in Yazd.

**Conclusion:** Sexual health literacy was positively correlated with sexual function and marital intimacy in women. It seems that increasing health literacy has led to increased knowledge about sexual physiology and sexual function, which can affect sexual satisfaction. Improvement of sexual health and appropriate interventions should be done in order to increase the level of health literacy. The results can be used in psychiatry counseling centers and women's health care centers.

**Keywords:** Sexual, Health literacy, Performance, Intimacy, Women.

## P-70

### Treatment suspension due to the coronavirus pandemic and its impact on the mental health of infertile people: A systematic review and meta-analysis of observational studies

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**Background:** Access to fertility treatments is a reproductive right; however, the COVID-19 pandemic

led to the widespread suspension of fertility treatments, potentially impacting the mental health of infertile individuals.

**Objective:** This study aimed to systematically analyze the prevalence of anxiety, depression, and stress among infertile individuals during fertility treatment suspension due to the COVID-19 pandemic.

**Materials and Methods:** This study adhered to the meta-analysis of observational studies in epidemiology guidelines. A comprehensive search was conducted in the Web of Science, PubMed, Embase, Scopus, and Cochrane library databases until December 31, 2022. All observational studies investigating the mental health outcomes, specifically anxiety, depression, and stress, of infertile individuals facing treatment suspension were included. Qualitative studies, editorials, commentaries, conference papers, and articles without full-text availability were excluded. The quality of studies was assessed by 2 researchers, independently, using the Newcastle-Ottawa scale. The random-effects model was applied to estimate the pooled prevalence. Meta-regression and subgroup analyses were performed to explore sources of heterogeneity.

**Results:** Of the 681 studies initially identified, 21 studies involving 5901 infertile participants met the inclusion criteria. Sixteen of these were included in the meta-analysis. The pooled prevalence of mental health issues in female participants was as follows: anxiety (48.4%, 95% CI [34.8-62.3]), depression (42%, 95% CI [26.7-59.4]), and stress (55%, 95% CI [45.4-65]). Furthermore, 64.4% (95% CI [50.7-76.1]) of participants expressed a desire to resume fertility treatments despite the ongoing pandemic.

**Conclusion:** The suspension of fertility treatments due to the COVID-19 pandemic negatively affected the mental health of infertile couples. It is crucial to ensure continuity in fertility care, with particular attention to the mental health needs of individuals experiencing infertility, even during a global public health crisis.

**Keywords:** Anxiety, Assisted reproductive technology, Covid-19, Depression, Infertility, Mental health, Stress, Meta-analysis.

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## P-71

### **Artificial intelligence in oocyte and embryo development: A new era in reproductive medicine**

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Artificial intelligence (AI) has emerged as a transformative force in various fields, including

reproductive medicine. Its application in oocyte and embryo development holds immense potential to revolutionize assisted reproductive technologies (ARTs) and improve pregnancy outcomes. AI-powered image analysis systems can objectively assess oocyte quality by analyzing morphological features, such as size, shape, and cytoplasmic texture. These systems can identify oocytes with the highest developmental potential, leading to improved selection for fertilization. Time-lapse imaging coupled with AI algorithms enables precise monitoring of embryo development. By analyzing key morphological parameters and developmental kinetics, AI can accurately predict embryo viability and implantation potential. This information empowers clinicians to select the most promising embryos for transfer, increasing the chances of successful pregnancy. AI can optimize various aspects of ART procedures, including: personalized treatment plans: by analyzing patient data, AI can tailor treatment protocols to individual needs, maximizing success rates. Automation of routine tasks: AI-driven robotic systems can automate tasks like sperm selection, oocyte retrieval, and embryo transfer, reducing human error and improving efficiency. Predictive modeling: AI models can predict pregnancy outcomes based on a multitude of factors, including patient characteristics, embryo quality, and treatment protocols. This information can guide clinical decision-making and patient counseling. The integration of AI in oocyte and embryo development offers several advantages, including: improved accuracy: AI-powered systems can provide more objective and accurate assessments compared to traditional methods. Enhanced success rates: by selecting the best embryos for transfer, AI can significantly increase pregnancy rates. Reduced costs: automation of routine tasks can lower the cost of ART procedures. Personalized medicine: AI enables tailored treatment plans, optimizing outcomes for individual patients. However, challenges remain, including the need for large datasets, ethical considerations, and regulatory frameworks. As AI continues to advance, it is crucial to address these challenges and ensure the responsible and ethical application of this technology in reproductive medicine. In conclusion, AI is poised to revolutionize oocyte and embryo development, offering significant advancements in reproductive medicine. By leveraging the power of AI, clinicians can improve patient outcomes, optimize treatment protocols, and ultimately fulfill the dreams of parenthood for many couples.

**Keywords:** Artificial intelligence, Embryo, Reproductive outcome, Oocyte.

## P-72

### **The impact of artificial intelligence in reproductive medicine and infertility**

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**Background:** Infertility is the most common and challenging global health problem in couples. The advancement of technology has increased the rate of diagnosis and treatment of infertility. Today, one of the most innovative technologies in the world is artificial intelligence (AI), and medical science is no exception to this rule. Therefore, it is one of the applications of AI in reproductive and infertility medicine. This article examines how AI can be effective in the diagnosis and treatment of infertility. Narrative research in medical databases (PubMed, Google Scholar, SID, Web of Science, MEDLINE and Cochrane) with the following keywords in Persian and English equivalents was performed: "Artificial intelligence", "Infertility", "Midwifery", "Reproductive Health". The search was conducted from 2010-2024 and among 30 relevant articles, after review 10 articles were selected. Research has shown that infertility can have several causes in men and women, each of which requires specialized investigations. The type of diagnostic and treatment methods play a significant role in solving the infertility problem of couples. Therefore, AI as a new idea can be used to understand, recognize, learn, and make decisions. It can act similarly to human intelligence and can even be faster, easier and more efficient. Among the applications of AI in infertility problems, we can mention the selection of the best egg and sperm cells to increase success, evaluation of embryo and egg quality before any intervention, prediction of the success rate of assisted reproductive technology, analysis of semen and interpretation of images. Since in today's societies the diagnosis and treatment of infertility are very important, especially in Iran, AI is expected to be developed in the field of education and counseling of infertile couples in addition to the field of diagnosis and treatment. So that it can be used in the field of fertility and health care. The introduction of AI in the community of midwives who are active in the field of reproductive health can create a new window in health care and help infertile couples benefit from other care in addition to therapeutic interventions.

**Keywords:** Artificial intelligence, Infertility, Midwifery, Reproductive health.

### P-73

#### Artificial intelligence and the future of infertility: Ethical and social challenges in the path of treatment

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**Background:** With the increasing prevalence of infertility in the world, the need for more accurate methods to assess this issue is clear. In recent years, the healthcare industry has utilized machine learning algorithms to improve the quality of care and efficiency. Artificial intelligence (AI) can play an effective role in diagnosing infertility and predicting the outcomes of assisted reproductive treatments.

**Objective:** This study aims to investigate the role of AI in infertility treatment, analyze its ethical and social challenges, and provide solutions to infertility treatment specialists for the intelligent and ethical use of new technologies.

**Materials and Methods:** In this review study, following the PRISMA guideline, a comprehensive literature search was conducted to identify relevant studies published between 2020 and 2024. The following databases were searched: PubMed, Science Direct, Google Scholar, and UpToDate. Search terms included the English keywords "Infertility", "Artificial Intelligence", "Social Challenge", "Ethical Challenge", and their Persian equivalents. Initially, 118 articles were retrieved. After removing duplicates, the remaining articles were screened based on their titles and abstracts to exclude irrelevant studies, resulting in 36 articles that met the initial inclusion criteria. Full-text versions of these potentially eligible studies were then obtained and assessed for eligibility based on predefined inclusion and exclusion criteria. A researcher-made checklist was used to select and review numerous quantitative and qualitative studies aligned with the aims of this study.

**Results:** Finally, 12 articles achieved the required quality score for inclusion in the final analysis. AI's application in infertility treatments has shown promising results across multiple areas. Studies have revealed that AI enhances the assessment of both male and female fertility, leading to more accurate evaluations of egg quality, endometrial receptivity, and sperm analysis. This includes the selection of superior embryos and optimizing the efficiency of egg stimulation. Furthermore, AI aids in making informed treatment decisions, contributing to higher fertility rates. It also streamlines treatment management, potentially lowering associated costs, and broadening access to care. Overall, AI's implementation appears to be a cost-effective solution. However, the integration of AI in medicine also poses significant ethical and legal challenges. Key among these are the availability of high-quality and extensive data sets necessary for training AI models. Standardizing medical images to ensure accurate analysis by AI systems is another crucial consideration. Protecting patient privacy during data usage is paramount, alongside continuous monitoring of AI model performance to guarantee safety and accuracy. Bias in training data, potentially leading to unfair outcomes, must also be addressed. Finally, it's essential to maintain the role of human decision-making, emphasizing that AI should support rather than replace the physician's expertise.

**Conclusion:** AI has great potential to revolutionize infertility treatment. Therefore, to achieve this potential, ethical and legal challenges must also be addressed. Responsible and informed use of AI, along with the development of standards and careful monitoring, can exploit the benefits of this technology in improving the health and care of couples with fertility problems.

**Keywords:** Infertility, Artificial intelligence, Challenge.

#### P-74

### The role of artificial intelligence in improving embryo selection for in vitro fertilization: A systematic review

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**Background:** The success of in vitro fertilization (IVF) treatments largely depends on selecting viable embryos for implantation. Traditional selection methods are based on morphological assessments that can lack predictive accuracy. Artificial intelligence (AI) has recently emerged as a tool to enhance embryo selection by providing more objective and precise assessments.

**Objective:** The aim of this study is to compare AI-based embryo selection methods with traditional morphological assessments in terms of their accuracy in predicting embryo viability, their impact on implantation success rates, the number of treatment cycles required, and the overall clinical outcome.

**Materials and Methods:** A systematic review was conducted in accordance with PRISMA guidelines. Databases such as PubMed and Google Scholar were searched using the following keywords: "artificial intelligence", "embryo selection", "implantation", "machine learning", and "in vitro fertilization". Included studies were peer-reviewed original human research using AI, machine learning, or deep learning for embryo selection in IVF, reporting outcomes such as implantation or pregnancy rates. Excluded were non-AI studies, reviews, animal research, and articles without full-text or outcome data. Studies employing deep learning algorithms to analyze images of embryos at various developmental stages were prioritized. Additional data, including participants' demographics and genetic markers, were considered when available to assess their impact on predictive outcomes.

**Results:** From a total of 412 retrieved articles, 76 duplicates were removed. After screening 336 articles by title and abstract, 52 were selected for full-text review. Finally, 18 studies met the inclusion criteria. Most of the included studies used deep learning or convolutional neural networks to evaluate embryo viability and predict implantation or live birth outcomes,

demonstrating improved accuracy compared to conventional scoring methods.

**Conclusion:** AI applications in IVF have substantial potential for optimizing embryo selection, offering a more precise, data-driven approach that could revolutionize reproductive medicine. By integrating AI into clinical IVF settings, success rates may be improved, ultimately providing a promising outlook for individuals seeking fertility treatments. However, further research is needed to standardize AI applications and assess their long-term efficacy in diverse clinical environments.

**Keywords:** In vitro fertilization, Embryology, Artificial intelligence.

#### P-75

### Innovative artificial intelligence applications in the diagnosis of women's infertility issues

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Infertility is a complex condition often resulting from a myriad of physiological and environmental factors. Recent advancements in artificial intelligence (AI) have emerged as transformative tools in the diagnosis and management of female infertility. This study examines the application of AI technologies in elucidating the etiologies of infertility, improving diagnostic precision, and tailoring treatment regimens. AI algorithms, with a particular emphasis on machine learning models, systematically analyze extensive datasets derived from clinical records, hormonal profiles, and imaging studies. These analyses facilitate the identification of patterns and correlations that may remain obscured through conventional diagnostic approaches. For instance, AI has shown promise in detecting conditions such as polycystic ovary syndrome and endometriosis by evaluating key biomarkers and patient histories, leading to earlier and more accurate diagnoses. Moreover, AI-driven predictive analytics can assess the likelihood of successful conception through various assisted reproductive technologies, enabling clinicians to tailor interventions based on individual patient profile. This personalized approach not only improves treatment outcomes but also reduces the emotional and functional burdens associated with infertility treatment. Ethical consideration surrounding AI in reproductive health, including data privacy and algorithm bias will also be addressed, ensuring that the implementation of these technologies aligns with patient care principles. The application of AI in diagnosis the causes of infertility

represent a significant advancement in reproductive medicine, offering hope for improved outcome and enhanced quality of life for women facing infertility challenges.

**Keywords:** Artificial intelligence, Infertility diagnosis, Machine learning, Reproductive health.

## P-76

### A deep learning-based method for detecting chromosomal abnormalities in microscopic images

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**Background:** Chromosomal abnormality is one of the major concerns in genetic disorders, which causes infertility and/or abortion. Traditional detection techniques, such as karyotype analysis, needs professional analysis and are time-consuming. Technological developments in image processing and deep learning present viable ways to automate this procedure.

**Objective:** This study aimed to develop a deep learning-based model for identifying chromosomal abnormalities, focusing on both numerical and structural anomalies, using convolutional neural networks (CNNs) and advanced image processing techniques. The goal was to provide a rapid and accurate method for diagnosing chromosomal abnormalities in genetic laboratories.

**Materials and Methods:** This work was a retrospective experimental analysis utilizing microscopic chromosomal imaging data. The dataset for this study consists of microscopic chromosome images, sourced from the Gel Cell Laboratory, including both normal and abnormal chromosome samples. Initially, a large dataset was used to train a deep learning model, and to address the class imbalance issue, data balancing techniques such as oversampling the minority class (abnormal images) and under-sampling the majority class (normal images) were applied. However, due to the complexity of the dataset, a CNN architecture was employed for the refined model. Additionally, a comparison was made with a pretrained ResNet-18 model to evaluate the performance differences. ResNet-18, being a well-known architecture for image classification tasks, was used as a baseline for assessing CNN's performance improvements. Following the balancing process, 2,832 images were selected for model training and optimization. Data augmentation techniques, such as rotation, brightness correction, and contrast adjustment, were applied to further enhance the dataset, ensuring better generalization of the model across diverse

scenarios. The improvements were achieved through the data balancing and augmentation techniques.

**Results:** The CNN-based model demonstrated strong performance in identifying chromosomal abnormalities, achieving 87% accuracy on the validation dataset and 85.03% accuracy on the test dataset. Precision and recall were 83% and 82%, respectively, indicating the model's ability to correctly identify abnormal chromosomes while minimizing false predictions. Data augmentation proved to be essential, particularly in handling low-quality images, and contributed significantly to the model's robustness across different image conditions.

**Conclusion:** In conclusion, the study demonstrates that chromosomal abnormalities can be effectively detected from microscopic images using deep learning, specifically CNNs combined with data augmentation techniques. The proposed model offers a reliable and efficient tool for genetic laboratories, facilitating faster and more accurate diagnoses of chromosomal disorders. Future work will involve enlarging the dataset and exploring transfer learning to further enhance model performance.

**Keywords:** Chromosomal abnormality, Deep learning, Convolutional neural networks, Genetic diseases, Karyotyping.

## P-77

### High intensity interval training along with nanomicelle curcumin improves assisted reproductive technologies outcomes in polycystic ovary syndrome women

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**Background:** Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women (4%-20%) when the ovaries create abnormally high levels of androgens, the male sex hormones that are typically in women in trace amounts. Therefore, finding a potential candidate for treating PCOS is necessary. Curcumin is a major active natural polyphenolic compound derived from turmeric (*Curcuma Longa*).

**Objective:** We aimed to investigate the effects of a high intensity interval training (HIIT) course along with nano micelle curcumin (NMC) supplementation on the serum levels of testosterone, luteinizing hormone) follicle stimulating hormone (FSH), egg maturation rate, embryo quality in infertile women with PCOs under intracytoplasmic sperm injection.

**Materials and Methods:** In this randomized clinical trial, 40 PCOs infertile women who referred to Jihad University Infertility Center, Qom, Iran for ICSI were

randomly divided in 4 groups (n = 10/each) of HIIT, HIIT with NMC, NMC, and control (without any intervention). The interferences began approximately 3 months before the start of the intracytoplasmic sperm injection cycle. Exercise training was 3 times a week and the supplement dose was 80 mg/day, 3 days a week to check the studied factors before and after 3 months of exercise intervention and NMC supplement, blood samples were taken and stored at 70°C. Also, on the day of puncture, the oocytes were checked for maturity and 72 hr after the microinjection, the quality of the embryos was checked and compared between groups.

**Results:** Our data showed that the amount of luteinizing hormone in the intervention groups has decreased statistically significantly (HIIT:  $p = 0.011$ , HIIT + NMC:  $p = 0.005$ , NMC:  $p = 0.013$ ). The FSH and testosterone levels increased significantly in the NMC and HIIT + NMC groups. FSH (NMC:  $p = 0.001$  and HIIT + NMC:  $p = 0.002$ ). Testosterone (NMC:  $p = 0.001$  and HIIT + NMC:  $p = 0.026$ ). The results showed that there was no statistically significant difference in the average number of oocytes obtained in the studied groups. Also, the results showed that the number of mature oocytes in the NMC and HIIT + NMC groups had a statistically significant increase compared to the 2 HIIT and control groups ( $p = 0.040$ ), in contrast to the average number of immature oocytes in the NMC and HIIT + NMC compared to the control group, a significant decrease was observed ( $p = 0.030$ ). The number of embryos obtained in the treatment groups has increased significantly compared to the control group, and the number of embryos in the NMC and HIIT + NMC groups is also higher than in the exercise group ( $p = 0.010$ ). The fertility rate has also increased significantly in the treated groups compared to the control group ( $p = 0.003$ ). The number of high-quality embryos in the NMC and HIIT + NMC groups showed a significant increase compared to the control group ( $p = 0.000$ ). At the same time, an increase was also observed in the exercise group, but it was not statistically significant compared to the control group.

**Conclusion:** NMC treatment and HIIT exercises can modulate hormones and assisted reproductive techniques outcomes in PCOs infertile women.

**Keywords:** Polycystic ovary syndrome, High intensity interval training, Nanomicelle curcumin, Assisted reproductive techniques.

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## P-78

### Genetically modified autologous stem-cell transplantation for endometrial dysfunctions

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Stem cells have become one of the safe and novel approaches for infertility due to their proliferative ability and differentiation. Also, molecular signaling and exogenous stimulators can control stem-cell metabolism, proliferation, and differentiation. Genetically modified stem cells are stem cells that have been changed to have specific traits as part of a treatment plan that improves how they respond to signals and controls their gene activity. Endometrial dysfunctions can lead to infertility due to abnormal endometrial thickness, disoriented endometrial proliferation, endometriosis, and low endometrial receptivity. This study aims to investigate a new approach to female infertility. Gene modifications on autologous stem cells are discussed and compared to non-modified autologous stem cells and other conventional approaches for endometrial dysfunctions. The study provides a literature review on recent investigations in the field of cell therapy for endometrial dysfunctions. Recent and valid studies from PubMed, Embase, and Scopus databases have been chosen and criticized to solve the problem and report results. Therapeutic outcomes for endometrial dysfunction using genetically engineered mesenchymal stem cells are shown. Reduction in fibrous formation, angiogenesis and endometrial proliferation are the histologic manifestations of modified mesenchymal stem cell transplantation by means of differentiation, proliferation, and enhanced cellular and molecular signaling response, including janus activated kinase (JAK)/ signal transducer and activator of transcription (STAT), inositol triphosphate (IP3), G-proteins, and growth factors. Compared to non-modified stem cells, there are better results for therapeutic efficacy. For endometrial dysfunction, modification of autologous bone marrow stem cells with a cardiotrophin-1 gene can be transplanted and shows improvement in neovascularization, enhanced proliferation, migration, and tube formation in vitro, and increased embryo receptivity in vivo based on upregulation of the JAK/ phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/STAT3 pathway. Modified menstrual blood mesenchymal stem cells also promote endometrial angiogenesis and proliferation. Animal studies also reveal endometrial lesion regression in mice with FMS-like tyrosine kinase (*Flt-1*) gene-modified endometrial stem cell transplantation, which lowers angiogenesis and enhances endometriosis. Viral vectors mediate all of the cell treatments mentioned. Genetically modified mesenchymal stem cells are considered new cell therapy approaches for infertility due to endometrial dysfunction. However, few human studies on gene modifications of stem cells limit the results. Thus, other types of stem cells, comprising embryonic and pluripotent stem cells, should be experimented with and may be a future perspective in this field.

**Keywords:** Genetically modified, Stem cell, Endometrial dysfunction, Infertility, Bone marrow.

## P-79

### Long-term psychological effects of social egg freezing in women who do not pursue pregnancy with preserved oocytes: A scoping review

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**Background:** Social egg freezing (SEF) has emerged as a reproductive strategy for women aiming to preserve fertility for non-medical reasons. While the procedure offers perceived biological and social advantages, the long-term psychological implications, particularly for women who do not use their cryopreserved oocytes, are underexplored. Initial emotional responses often include relief and empowerment, but over time, some women may experience regret, uncertainty, or dissatisfaction. This underscores the importance of understanding the long-term psychosocial effects of SEF.

**Objective:** This scoping review evaluates the literature on the long-term psychological effects of SEF in women who do not attempt pregnancy using their preserved oocytes.

**Materials and Methods:** A systematic search was conducted across major databases, including PubMed, Scopus, and Web of Science, using predefined criteria and MeSH terms to identify studies focused on women who underwent elective SEF and did not use their frozen oocytes, with a long-term follow-up of at least 1 yr post-freezing. Data extraction and thematic synthesis were performed in accordance with PRISMA-ScR guidelines to map key findings and research gaps. Eligible study types included quantitative, qualitative, mixed-methods studies, reviews, and relevant case series. Only English-language articles were considered, with no restrictions on publication date. Studies related to medical egg freezing, those involving oocyte use, studies lacking psychological data, short-term follow-up, non-scholarly articles, editorials, and non-English publications (if translation was not feasible) were excluded. The study selection, data extraction, and thematic synthesis processes were independently carried out by 2 reviewers.

**Results:** A total of 300 records were identified (PubMed: 120, Scopus: 85, Web of Science: 95); after removing 110 duplicates, 190 records were screened. Following title/abstract review and full-text assessment, 15 studies met the inclusion criteria and were included in the scoping review. Key findings were categorized into 2 themes: 1) evolving emotional states: many women initially experienced relief and empowerment from egg freezing; however, psychological responses often evolved, with some expressing regret or uncertainty while others reported sustained satisfaction. 2) awareness and expectations: women frequently cited inadequate pre-procedural counselling regarding the

likelihood of oocyte utilization, contributing to diverse emotional outcomes.

**Conclusion:** The psychological effects of unused SEF are complex and multifactorial, influenced by personal and contextual dynamics. While many women derive a sense of control and reduced reproductive pressure, a notable subset experiences negative psychological consequences, emphasizing the critical need for comprehensive counseling and post-procedural support. Further longitudinal research is essential to deepen understanding and optimize this population's clinical and psychosocial care strategies.

**Keywords:** Pregnancy, Egg freezing, Scoping review, Psychological.

## P-80

### Chronic administration of methadone induces structural changes in the ventral prostate of Wistar rats

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**Background:** Methadone is a synthetic opioid frequently utilized in addiction treatment and has been demonstrated to influence several physiological systems. However, its effects on prostate health are not well understood.

**Objective:** This study investigated the impacts of methadone and opium tincture on the morphology of prostate tissue.

**Materials and Methods:** In this experimental study, 18 adult male Wistar rats, (8-10 wk, 200 ± 20 gr) were employed. The subjects were randomly assigned to 3 equal groups (n = 7/each): control, opium tincture, and methadone syrup. Throughout the study, the control group received 1 cc of normal saline orally once daily for 9 wk. The rats in the opium tincture and methadone syrup groups were initially administered escalating doses of opium and methadone orally during the 1<sup>st</sup> wk. Following this, they were treated with the maximum doses of opium tincture (150 mg/kg) and methadone syrup (16.25 mg/kg) for an additional 8 wk. Upon completion of the treatment period, the animals were euthanized, and blood and prostate samples were collected for analysis of testosterone concentration and histological evaluation.

**Results:** In comparison to the control group, the prostate weight index (p < 0.001) and serum testosterone concentration (p < 0.05) were significantly reduced solely in the group receiving opium tincture. Additionally, treatment with opium tincture led to a significant decrease in the epithelial height of the ventral prostatic acini (p < 0.001). In contrast, methadone treatment resulted in a significant increase in epithelial

height ( $p < 0.01$ ). Moreover, a notable increase in stromal space within the ventral prostate acini was observed in the methadone-treated group, indicating enhanced cellular proliferation in this region.

**Conclusion:** Prolonged administration of methadone syrup increased the proliferation of both prostatic stromal and epithelial cells. These structural and morphometric alterations may represent potential risk factors for developing adverse remodeling processes in the ventral prostate of rats.

**Keywords:** Methadone, Addiction medicine, Prostate, Testosterone, Rats.

## P-81

### Global, regional, and national prevalence and disability-adjusted life years for primary infertility in Iran and provinces of Iran: Results from the global burden of disease study, 1990-2021

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**Background:** Primary infertility refers to the inability of a couple to achieve a pregnancy after at least 12 months of regular, unprotected sexual intercourse, provided they have never conceived a child before.

**Objective:** In this study, the results of the Global Burden of Disease (GBD) 2021 are presented and an assessment of current trends in the burden of primary female and male infertility diseases 1990-2021 at the global, regional and national levels and all provinces of Iran is presented.

**Materials and Methods:** This cross-sectional study utilized publicly available data from the GBD study to examine the point prevalence and years lived with disability (YLDs) at the global, regional, national, and provincial levels in Iran between 1990 and 2021. The analysis included point prevalence, YLD counts, and rates per 100,000 populations, all reported with their 95% confidence intervals. Trends were evaluated for 1990 and 2021, with provincial-level comparisons in Iran.

**Results:** Global: in 2021, age-standardized point prevalence was 732.1 (95% UI: 363.2-1329.8) in women and 426.9 (189.9-827.6) in men, reflecting increases since 1990 (women: 577.2; men: 344.0). Iran: prevalence remained high, with 1237.8 (596.4-2365.4) in women and 843.4 (380.9-1672.8) in men in 2021. Provincial disparities were observed, with highest prevalence in Khuzestan, Ilam, and Mazandaran (women) and Isfahan, Yazd, and Markazi (men). YLDs: Iran's rate (7.8; 2.8-19.2) exceeded the global average (4.3; 1.6-9.9) in 2021.

**Conclusion:** Primary infertility prevalence and YLDs in Iran were substantially higher than global and MENA

region averages, with persistent provincial inequalities. While the analysis focused on 1990 and 2021 as benchmark years, further research is needed to explore interim trends and drivers.

**Keywords:** Infertility, Global burden of disease, Prevalence, Years lived with disability.

## P-82

### Novel endometrioma model causes infertility via cystic lesions, histopathologic, and hormonal changes in mice

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**Background:** Endometriosis is characterized by the presence of tissue comparable to the endometrium found outside the uterus, affecting 5-10% of women during their regenerative a long time. Although the torment side effect is one of the most common signs of this gynecologically common malady, up to 50% of women with endometriosis endure from infertility. In expansion to the anatomical obstructions, altered ovarian function in women with endometriosis may be attributed to fertility. Ovarian function can be divided into three essential components: 1) the secretion of ovarian hormones, 2) the support of follicular development from the preantral stage to the postovulatory luteal stage, and 3) the storage of dormant oocytes. Endometriosis can affect ovarian functions in different ways. Here, the affiliations between ovarian functions and endometriosis are talked about together with current prove.

**Objective:** Our objective was to create a mouse model for ovarian endometriosis and assess how endometriosis impacts ovarian tissue macroscopically, microscopically, and hormonally.

**Materials and Methods:** This experimental study included three groups: control (in this group, the mice remained intact), sham (in this group, surgery was performed without any treatment), and model, each consisting of 4 NMRI mice (6-8 wk, 25-30 gr). After performing bursectomy in the proestrus stage, endometriosis lesions were created in the model group by suturing 5×5 mm pieces of uterine tissue onto the ovarian surface. All surgeries were performed in the proestrus phase. After maintaining controlled conditions for 4 wk, blood samples were collected from the mice for comparison with the control group. Furthermore, the mean weight of lesions in the model group was compared to the control and sham groups. Subsequently, the generated ectopic tissue and the ovary were analyzed for histological assessment using hematoxylin-eosin

staining. Furthermore, Masson's trichrome staining, along with Image J software, was utilized to examine and quantify the fibrotic tissue area.

**Results:** Out of four tissue samples sewn onto the ovarian surface, histopathology confirmed three cases of endometriosis lesions (75%). The average weight of the lesions and the concentration of 17 $\beta$  estradiol in the model group were noticeably higher than those in the control group ( $p < 0.05$ ). The model group exhibited the development of endometrial glands and stromal cells. The extent of fibrotic regions in the model group was considerably greater than in the control group ( $p < 0.05$ ).

**Conclusion:** Our findings indicated that epithelial and mesenchymal stromal cells found in a piece of a mouse's uterine horn can infiltrate the ovary and form a model for endometriosis. Additionally, this model offers a chance to assess agents or methodologies to combat ovarian endometriosis within a preclinical framework.

**Keywords:** Endometrioma, Mouse model, 17 $\beta$  estradiol, Proestrus phase.

### P-83

#### Novel technologies for the treatment of infertility with mesenchymal stem cells as a bio organ

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Inability to conceive after having regular unprotected sex for more than 12 months is defined as infertility and can be due to male, female, or both fertility problems, but sometimes the cause of infertility is unknown. Infertility as one of the most common troubles in the world is affecting approximately 15% of couples worldwide. It has negative consequences on society and infertile individuals, and its prevalence has increased all over the world for various reasons. In this review study, a search was conducted using international databases (Medline/PubMed, Scopus, and ISI/Web of knowledge), and national databases (Scientific Information Database, MagIran, IranMedex, and IranDoc) from 2015-2024. Subsequently, Persian and English language papers referring to "Cell therapy" and "infertility" were included. Stem cells release a variety of bioactive factors, which can either remain freely soluble or be encapsulated within extracellular vesicles such as exosomes and microvesicles. The therapeutic application of mesenchymal stem cell (MSC) secretions -referred to as conditioned media (CM)- or purified extracellular vesicles from MSCs has demonstrated comparable benefits to direct stem cell transplantation in various disease models, enhancing functional recovery. Accordingly, in recent years, there has been an increasing focus on its treatment. Many infertile couples (80%) can be cured with recent advances in assisted reproductive technology. However, many couples are unable to conceive despite using assisted reproductive

technology. Recently, stem cells have been considered therapeutic targets for numerous diseases, including infertility, due to their self-renewal and differentiation potential into almost any cell type, and they can even correct genetic disorders in children. Therefore, clinical researchers have investigated the therapeutic use of MSCs in infertility. In general, the extensive clinical application of MSCs is considered a hopeful option in the development of stem cell-based infertility therapies. Reliable success has been achieved in the differentiation of stem cells into germ cells, and clinical trials of transdifferentiated MSCs may provide valuable results to combat the increase in infertility in the world. In general, the start of clinical trials and the use of MSCs in infertility clinics can promise new treatment horizons for infertile couples.

**Keywords:** Infertility, Mesenchymal stem cells, Transdifferentiation, Assisted reproduction technology.

### P-84

#### In vitro gametogenesis: Ethical considerations and future perspectives

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In vitro gametogenesis (IVG) is a novel biomedical technology that has succeeded in the conservation of endangered species and in accelerating the genetic selection of livestock. Also, one of the most distinguished innovations is the role of IVG in human infertility. However, developing IVG for human infertility is still experimental. IVG is a complicated method that manages infertility through the molecular mechanism of germ cells, embryonic stem cells, and pluripotent stem cells, which can be the origin of in vitro-derived gametes. IVG reduces oocyte donation's psychological and physical challenges, enables women to be fertile in advanced ages and even post-menopausal fertility, and helps people find appropriate oocytes or embryos (in genetic features). In addition to several advantages, some challenges include moral, legal, social, and ethical concerns. The undefined concept of parenthood especially in same-sex partners, single individuals, and even deceased ones, misuse of IVG in eugenics and artificial baby production, ignoring the authority of patients with infertility and justice, and increased risk of genetic mutations in a child's future are some adverse applications of IVG which lead to ethical challenges. IVG emerges as an attractive therapeutic approach to infertility and other reproductive disorders. However, due to the wide range of its developments, even for non-therapeutic procedures, many ethical issues arise based on the cultural and religious background of different societies. To fully benefit from IVG's potential, developing some guidelines for IVG

applications is essential. In this regard, standard protocols avoid misuse and adverse effects and improve therapeutic outcomes.

**Keywords:** Gametogenesis, IVG, Ethics, Infertility.

## P-85

### Impact of stem cell therapy by stromal vascular fraction on reduction of testicular injury

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The utilization of stem cells can reduce testicular damage and enhance the generation of sperm. Adipose-derived stromal vascular fraction (SVF) can be easily obtained without the need for in vitro expansion, potentially reducing the risks associated with xenogenic nutritional foundations, bacterial contamination, and other factors related to cell culture. The purpose of the current review is to investigate the impact of SVF on reduction of testicular injury. This scientific review as titled impact of stem cell therapy by SVF on reduction of testicular injury explored several studies. These were searched in scopus, Pubmed and Web of science by applying keywords included SVF, testicular injury and stem cell therapy in both combinations of OR and AND. Time frame of related articles was from 2021-2024. The language of all of articles was English. Using autologous uncultured SVF can help protect the testis from injury caused by testicular ischemia-reperfusion and support spermatogenesis, offering important clinical implications for preventing infertility resulting from testicular torsion-detorsion. In a study investigating the use of bone marrow mesenchymal stem cells (BM-MSCs) as a new approach against doxorubicin-induced toxicity in rat testes, the results demonstrated that BM-MSCs significantly reduced testicular oxidative stress by lowering malondialdehyde levels and enhancing antioxidant capacity. Histologically, the damage caused by doxorubicin, including testicular atrophy, severe spermatogenesis impairment, and reduced diameter and thickness of the seminiferous tubules, was notably improved following BM-MSC administration. Additionally, the effects of SVF on busulfan-induced testicular damage in rats indicated that SVF alleviated oxidative stress in both testis tissue and serum. Injecting SVF into damaged testicular tissue led to an increase in healthy spermatozoa numbers and a decrease in unusual tail numbers.

**Keywords:** Stromal vascular fraction, Testicular, Injuries, Stem cells, Therapy.

## P-86

### Engineering innovations and biotechnological advances in addressing infertility due to uterine cancer

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Infertility is a significant concern for women diagnosed with uterine cancer, as the disease and its treatments can severely impact reproductive health. This literature aims to explore cutting edge engineering and biotechnology solutions that address infertility resulting from uterine cancer. Recent advancements in reproductive technologies such as in vitro fertilization and cryopreservation have shown promise in preserving infertility in women undergoing cancer treatments. Since the structure and function of uterus is complex, applied uterine tissue engineering requires highly specialized biomaterials with natural extracellular microenvironment. Innovative biomaterials and tissue engineering techniques are being developed to reconstruct the uterine environment, potentially restoring fertility post-treatment. In our research the role of hormonal therapies and fertility preservation strategies that minimize the risk of cancer recurrence will be focused. We will also discuss the ethical implications and patient-centered approaches in the application of these technologies, ensuring that woman's reproductive rights and choices are prioritized. Furthermore, interdisciplinary collaboration between oncologists, reproductive specialists, and bioengineers will be highlighted, showcasing how integrated efforts can lead to improved outcomes for women facing infertility due to cancer.

**Keywords:** Infertility, Uterine cancer, Biotechnology, Reproductive health, Tissue engineering, IVF.

## P-87

### Protective effect of crocin on steroidogenesis disturbances in human granulosa cells of normal and polycystic ovaries

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**Background:** Estradiol and progesterone are the main steroid hormones that may play essential roles during the follicular and luteal phases of the menstrual cycle, oocyte fertilization, and embryo implantation. The granulosa cells of women with polycystic ovary syndrome (PCOS) fail to increase the expression of aromatase and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD), causing markedly decreased estrogen and

progesterone secretion. Crocin, a main component of saffron, has been reported to act as a regulator of some reproductive pathways.

**Objective:** In this study, the role of crocin was compared in regulating the endocrine function of granulosa cells in women with normal and polycystic ovaries.

**Materials and Methods:** This experimental study was performed on ovarian granulosa cells (GCs) of 40 women who were referred to Shiraz Ghadir Mother and Child Hospital, Shiraz, Iran between November 2023 and August 2024 for in vitro fertilization or intracytoplasmic sperm injection. Control (normal) group (N-GCs) consisted of 20 women with healthy ovaries diagnosed as male factor, tubal disease, unexplained infertility, as well as egg donors or patients with normal reserve of the ovary. Case (PCOS) group (PCO-GCs) consisted of 20 women with PCOS that their syndrome was diagnosed by a gynecologist according to Rotterdam criteria. Inclusion criteria were 18-35 yr old and a body mass index between 18.5-30 kg/m<sup>2</sup>. Participants were excluded from the study if they had major medical disorders (such as other hyperandrogenism states, thyroid disorders, endometriosis, chronic metabolic syndrome, and diabetes) or if they were cigarette smokers and alcohol consumers. Follicular aspirates included GCs collected from dominant follicles on the day of oocyte retrieval. The GCs were cultured in Dulbecco's modified Eagle's medium/F12 (DMEM-F12) medium. To study the effect of crocin on the aromatase and 3 $\beta$ -HSD activities, GCs (4 $\times$ 10<sup>6</sup> cells) were divided in half and then cultured in 6-well plates for 48 hr with or without crocin (200  $\mu$ M). The media were collected to measure estradiol and progesterone as indicators of aromatase and 3 $\beta$ -HSD activity.

**Results:** Basal estradiol ( $p = 0.039$ ) and progesterone ( $p = 0.025$ ) levels of the PCOS group were significantly lower than the normal group. In the crocin-treated GCs group, the total level of estradiol and progesterone at 48 hr were increased compared to the untreated-GCs group ( $p < 0.0001$ ). However, treatment with crocin did not return the production of sex hormones to normal levels.

**Conclusion:** Our results showed that ovarian aromatase and 3 $\beta$ -HSD activities in PCOS were impaired relative to healthy women. An increase in aromatase and 3 $\beta$ -HSD activities characterizes the stimulatory effect of crocin. This effect on steroidogenic enzyme activity supports crocin's post-transcriptional modulation of these enzymes.

**Keywords:** *Estradiol, Crocin, Progesterone, PCOS, Granulosa cells.*

## P-88

**How does serum starvation affect the morphological, biochemical, and content heterogeneity of exosomes extracted from human adipose-derived mesenchymal stem cells?**

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**Background:** Exosomes, extracellular vesicles with diameters ranging from 40-160 nm. According to the size and content heterogeneity, they can be classified into Exo A (40-75 nm, CD63 biomarker, protein context "X"), Exo B (75-100 nm, CD9 biomarker, protein context "Y"), and Exo C (100-160 nm, CD81 biomarker, protein context "Z").

**Objective:** This study investigated the effects of serum starvation on the morphological characteristics, biochemical properties, stability, aggregation, and protein concentration of exosomes derived from adipose tissue mesenchymal stem cells (AD-MSCs).

**Materials and Methods:** In the experimental study, 7 human adipose tissues were collected from healthy women by selective liposuction at Gandhi Hotel Hospital, Tehran, Iran. Adipose tissue-derived AD-MSCs were isolated and confirmed using flow cytometry and differential staining. The confirmed AD-MSCs were cultured under serum starvation (1% knock-out serum) and control (1% knock-out serum) conditions for 24 hr at 37°C, 5% CO<sub>2</sub>, 80% humidity, and 22% O<sub>2</sub>. The effect of serum concentration on apoptosis and differentiation was detected using acridine orange, alizarin red, oil red O, and toluidine blue staining. A scratch assay evaluated the migration of AD-MSCs under both conditions. Then, exosomes were isolated from both groups using ultracentrifugation (300x g/10 min, 2000x g/10 min, 10000x g/30 min, and 100000x g/70 min) and analyzed by scanning electron microscopy, fourier-transform infrared spectroscopy, transmission electron microscopy, dynamic light scattering), zeta potential, and BCA.

**Results:** Serum starvation does not affect the survival rate (apoptosis) and differentiation potential of AD-MSCs into chondrocytes, osteocytes, and adipocytes ( $p = 0.059$ ). However, AD-MSCs in the serum starvation condition demonstrated higher migration ability compared to the control group ( $p = 0.025$ ). The expression of the CD63 molecular marker in the extracted exosomes from AD-MSCs under serum starvation was 60.4%. We successfully obtained Exo A in the serum starvation condition based on the biochemical and morphological characteristics of exosomes. DLS analysis showed that 95% of exosomes from the serum starvation group measured  $48.9 \pm 5.4$  nm with the highest frequency at 47.7 nm; (mode: 47.7 nm), while 72% of nanoparticles in the control group were  $350.2 \pm 43.6$  nm with the highest frequency at 339.8 nm ( $p = 0.031$ ). Polydisperse intensity in the serum starvation and control groups were 0.492 and 0.727, respectively ( $p = 0.028$ ). Transmission electron

microscopy and zeta potential analyses indicated improved dispersion, stability and uniformity of exosomes in the serum starvation group ( $p = 0.044$ ). Zeta potentials in the serum starvation and control groups were  $-9.0$  mV and  $-1.0$  mV, respectively ( $p = 0.035$ ). FTIR analysis revealed subtle changes in biochemical composition, with higher protein contents in the serum-starved exosomes ( $p = 0.074$ ). Bradford analysis demonstrated 51.399% and 21.754% total protein concentration in the serum starvation and control groups, respectively ( $p = 0.031$ ).

**Conclusion:** Serum starvation not only improves the morphological, biochemical and functional characteristics of exosomes, but also leads to the isolation of Exo A.

**Keywords:** Mesenchymal stem cells, Exosomes, Serum starvation.

### P-89

#### Revolutionizing male fertility solutions: 3D printing artificial testes with testicular tissue-derived bio-inks

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Testicular scaffolds are categorized as an innovative approach for fertility preservation in individuals at high risk of reproductive loss due to medical conditions or treatments. Recent advances in tissue engineering, particularly in bioinks and 3D bioprinting, show promise in creating functional artificial testes. The engineered structures can restore sperm production by mimicking the natural testicular microenvironment. As male infertility rates rise, exploring such biotechnological solutions becomes increasingly urgent. This study aimed to evaluate the potential of bioinks in creating functional artificial testes that may restore sperm production. The study analyzed studies conducted since 2020 using electronic databases like PubMed, Scopus, and Web of Science, focusing on research studies of any design as well as Persian and English written papers. Keywords included "Artificial testes", "3D printing", and "testicular tissue-derived bio-inks". Observations have shown that 3D bioprinting of alginate-based scaffolds is feasible in vitro. Reports indicate that after bioprinting the fabricated scaffolds and culturing testicular cells on the scaffolds, there is a difference between number of spermatogonia stem cells with the control group, indicating the creation of the desired model. The experimental results demonstrated that 3D-printed scaffolds made with 5% Tissue Extracellular Matrix (T-ECM) had a uniform surface structure in both in vitro and in vivo environments, exhibiting strong cell attachment and high biocompatibility. Also, recent research has focused on

the use of decellularized extracellular matrix bioinks to develop artificial testes, which was accompanied by significant results. All in All, it can be stated that reproductive bioengineering is advancing with the intention to develop biomaterials to restore male fertility and address the increasing cases of male infertility as well. Techniques like 3D bioprinting and microfluidic systems are innovative for the development of testicular organoids which can mimic the testicular microenvironment. In this context, 3D printing artificial testes can decrease the need for experimental animals in research on spermatogenesis.

**Keywords:** 3D printing, Artificial testes, Bio-inks, Male fertility.

### P-90

#### Morphological, chemical, and functional characteristics of nanoparticles derived from human placental extract, applications in regenerative medicine

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**Background:** Several studies have shown the effect of placenta extract on the immune system, inflammation, oxidative stress, skin rejuvenation, and treatment of acute and chronic wounds, alopecia, psoriasis, and vitiligo.

**Objective:** We aimed to successfully isolate and characterize the nanoparticles from the human placenta for applications in tissue engineering and regenerative medicine.

**Materials and Methods:** In the descriptive study, 5 human placentas were collected from healthy women at Gondhi Hotel Hospital, Tehran, Iran. Inclusion criteria included ages 35-45 yr, full-term pregnancies (38-40 wk) with no history of drug addiction, metabolic diseases, liver disorders, and mycoplasma, bacterial, fungal, and viral infections. After macroscopic and microscopic evaluation of placenta, we separated the chorionic villi and digested them using mechanical and enzymatic digestion (trypsin and pepsin). The digested suspension was filtered through a  $70 \mu\text{m}$  mesh, centrifuged at  $300\times g/10$  min,  $2000\times g/10$  min,  $10000\times g/30$  min, and  $100000\times g/70$  min, and then lyophilized. Nanoparticles in the placenta extract were analyzed using scanning electron microscopy, fourier-transform infrared spectroscopy, transmission electron microscopy, dynamic light scattering, and zeta potential. Total protein concentration was also detected using the

Bradford test, and the superoxide dismutase was evaluated by calorimetric method.

**Results:** Nanoparticles extracted from the chorionic placenta exhibited molecular markers CD63, CD9, and CD81 with the expression of 60.4%, 65.4%, and 34.1%, respectively. dynamic light scattering analysis demonstrated that 87% of nanoparticles in the placenta extract had a diameter of  $121.7 \pm 19.9$  nm, with the highest frequency of nanoparticles at 124.5 nm; mode: 12.45 nm). Meanwhile, 13% of nanoparticles measured  $32.5 \pm 2.8$  nm, with a mode of 124.5 nm). The Z-average and polydispersity index of extracted nanoparticles in placenta extract was 92.8 nm and 0.349, respectively. transmission electron microscopy and SEM analyses showed that the nanoparticles in the placenta extract had a spherical, hollow, and homogeneous morphology with uniform distribution. The zeta potential of nanoparticles derived from placenta extract was 34 mV, indicating stability. These nanoparticles remained stable without electrostatic fluctuations over time. The protein concentration in the placenta extract was 28.274%, and the superoxide dismutase antioxidant activity was  $34 \pm 0.30$  U/mg. FTIR analysis identified various functional groups in the placenta extract, including amide I and II peaks, C-O peaks, and P = O peaks.

**Conclusion:** Nanoparticles derived from human placenta extract can be effectively used to accelerate wound healing in tissue engineering and regenerative medicine.

**Keywords:** Placenta, Nanoparticles, Regenerative medicine, Tissue engineering.

## P-91

### Bioactive glass incorporated with zinc oxide for regenerative medicine

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Bioactive glass (BG) and zinc oxide (ZnO) have unique properties, but their combination offers exciting possibilities for use in regenerative medicine by addressing key challenges in tissue repair, such as enhancing biocompatibility, angiogenesis, and antimicrobial properties. The synergy between BGs and ZnO has broadened their applications, from hard tissue to soft tissue regeneration. This review focuses on the properties of ZnO-doped BGs in soft and hard tissue regeneration, as well as their potential in reproductive tissue engineering. The study evaluates all the researches on ZnO-doped BGs. ZnO-doped BGs have

shown effectiveness in some pathways including: 1) ion release: controlled release of ions such as zinc, calcium, and silicon to support cellular activities like proliferation, differentiation, and matrix formation. 2) antibacterial: ZnO conveys strong antimicrobial properties by disrupting bacterial cell membranes and preventing biofilm formation, which are crucial for infection control. 3) anti-inflammatory: ZnO regulates inflammatory responses and promotes healing by reducing chronic inflammation at the site of injury. 4) angiogenesis: in some dosages, ZnO enhances the angiogenesis process which is crucial for healing. Briefly, BGs-doped ZnO conduct the following properties: 1) hard tissue regeneration: stimulating osteoblasts and enhancing vascularization in bone repair. 2) soft tissue regeneration: promoting fibroblast proliferation and angiogenesis, useful in wound healing, burns, and chronic wound treatment. 3) dental applications: preventing infections, supporting enamel and dentin repair, and promoting remineralization. 4) reproductive tissue engineering: female reproductive system: repairing uterine defects, supporting endometrial regeneration, and providing a matrix for ovarian cell growth (hormonal restoration and fertility preservation). 5) male reproductive system: testicular tissue regeneration and drug delivery for testicular disorders. 6) drug delivery: serving as carriers for hormones, growth factors, and antimicrobial agents in conditions like endometriosis and infections. Despite their promising potential, ZnO-doped BGs face challenges such as cytotoxicity at high ZnO concentrations, the need for improved production scalability, and a lack of long-term clinical studies to assess safety and effectiveness, particularly in reproductive tissues. Further research is necessary to optimize their use in regenerative medicine.

**Keywords:** Bioactive glass, Zinc oxide, Regenerative Medicine.

## P-92

### Temperature and pH sensitive lipid nanocarriers (liposome) containing Foeniculum vulgare essential oil and its potential bioactivity against ovarian cancer cell line

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**Background:** Ovarian cancer is one of the deadliest cancers among women, claiming a significant portion of lives each year. Additionally, these cancers can have a profound impact on pregnancy and fertility. Recently, the use of nanocarriers loaded with anticancer compounds has emerged as a promising strategy for cancer treatment, offering potential effectiveness with fewer side effects.

**Objective:** This study aim to design lipid nanocarriers encapsulating *Foeniculum vulgare* essential oil and evaluate their toxicity in both ovarian cancer (A2780 cell line) and human fibroblast (HFF) cell lines.

**Materials and Methods:** Initially, liposomal formulations (F1-F5) were prepared using different molar ratios of phosphatidylcholine and cholesterol along with *Foeniculum vulgare* essential oil. The encapsulation efficiency and release profile of the essential oil from each formulation were then evaluated. The formulation with the highest encapsulation efficiency and the best release profile was selected, and the physicochemical properties of the resulting nanoparticles, including zeta potential, particle size, and particle morphology, were investigated. In the next step, the toxicity of the selected formulation on the growth of ovarian cancer cells (A2780 cell line) and the HFF cell line was examined using the methyl thiazol tetrazolium (MTT) method. Finally, the entry of the nano-system into ovarian cancer cells was investigated. Finally, the physicochemical stability of the optimized nano-formulation was monitored for 120 days under refrigerated conditions ( $4 \pm 1^\circ\text{C}$ ) in light-protected containers. Particle size, zeta potential, and encapsulation efficiency were measured at 30-day intervals.

**Results:** Formulation F4 was selected due to its higher encapsulation efficiency (58.2%) and its slow, continuous release profile over 48 hr. The nanoparticles obtained from this formulation (F4) had a size of 120.6 nm and a zeta potential of -15.3 mV. Electron microscope images revealed that the surface of the nanoparticles was smooth and spherical, with no aggregation observed. MTT assay results demonstrated that the liposomal essential oil exhibited greater toxicity than the free essential oil against the A2780 ovarian cancer cell line  $p < 0.001$ , while showing negligible toxicity to the HFF cell line. Moreover, the IC50 values for the essential oil-loaded nanocarrier and free essential oil against the A2780 cell line were 51.9  $\mu\text{g/mL}$  and 59.3  $\mu\text{g/mL}$ , respectively. Furthermore, cell uptake studies indicated that the liposomal system containing the essential oil successfully entered ovarian cancer cells. Stability analysis of essential oil-loaded nanoliposomes revealed a 7.5% decrease in encapsulation efficiency, an 11.2 nm increase in particle size, and a 5.5 mV shift toward more positive zeta potential values over 120 days.

**Conclusion:** Considering the high prevalence of ovarian cancer and its detrimental effects on women's fertility and pregnancy, along with the numerous side effects associated with conventional cancer treatments, the use

of nanoliposomes containing *Foeniculum vulgare* essential oil as an antiproliferative agent is proposed for therapeutic research in ovarian cancer.

**Keywords:** Ovarian neoplasms, Cell line, Tumor, *Foeniculum*, Liposomes, Nanoparticles, Antineoplastic agents.

## 6<sup>th</sup> Congress of Reproductive Genetics

### P-93

#### Cytogenetic signatures of infertility: Report of 4105 infertile couples

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**Background:** Fertility refers to the ability to achieve a clinically successful pregnancy. More than 186 million people in the world suffer from infertility, and the reason for approximately 15% of couples experiencing infertility is unexplained. Chromosome abnormalities can significantly impact fertility and reproductive outcomes with increased miscarriage risk, birth defects, and assisted reproduction.

**Objective:** Here, we want to study infertile couples and their chromosomal aberrations.

**Materials and Methods:** This retrospective study analyzed 4,105 clinical profiles of infertile couples referred to the Yazd Reproductive Sciences Institute (Yazd, Iran) for comprehensive cytogenetic screening between April 2016 and March 2024. Prior to initiating assisted reproductive techniques, peripheral blood lymphocyte cultures were collected from participants for karyotype analysis using Giemsa-banding techniques and their cytogenetic map were evaluated.

**Results:** Analysis of the infertile karyotype screening results identified an increased incidence of chromosome abnormalities as a signature of genomic instability. The study revealed that from a total of 4105 samples, 3774 cases (91.94%) had a normal karyotype and 331 cases (8.06%) had chromosomal aberrations. These aberrations were found in 86 (25.98%) females and 245 (74.02%) males. The cases with different kinds of chromosome variants and infertility included 39 cases (11.78%) with structural aberrations, 122 cases (36.86%) with numerical abnormality, 9 case (2.72%) with complex abnormality ( $\geq 2$ ), and 161 cases (48.64%) with polymorphic variants.

**Conclusion:** The findings of this study underscore the importance of detecting chromosomal abnormalities in infertile couples, as it facilitates the elucidation of infertility etiology and informs the optimization of treatment approaches.

**Keywords:** Infertility, Cytogenetics, Karyotype, Chromosome aberrations.

#### P-94

### Investigating the effect of spirulina on the activation of Fas/Fas Ligand-Apoptosis signal-regulating kinase 1-p38 pathway in breast and prostate cancer cells

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**Background:** Sufferers with advanced or recurrent cancer have a poor prognosis; their 1-year survival rate is only 10-20%. Chemotherapy is considered the same old treatment for sufferers with advanced or recurrent cancer. However, resistance to treatment might also broaden, ensuing compromising the efficacy of the drug to treat advanced or recurrent cancer. It is widely known that regular intake of certain natural substances, so-referred to as chemopreventive agents, can lessen the risk of certain cancers. Consequently, spirulina is an attractive chemotherapeutic agent due to its useful health outcomes.

**Objective:** This study aims to determine the effects of spirulina on proliferation, apoptosis, nitric oxide secretion and the expression of genes related to apoptosis in breast and prostate cancer cells.

**Materials and Methods:** In this experimental study, a hydroalcoholic extract of spirulina was prepared. The 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide method was used to investigate the effect of the extract on the survival of breast cancer, prostate cancer and fibroblast cells. DNA fragment percentage and nitric oxide level were measured using spectroscopic methods. A real-time polymerase chain reaction test was used to analyze the Apoptosis signal-regulating kinase 1, p38, *Fas* and *Fas Ligand* gene expression of control and treated cells.

**Results:** After 24, 48, 72 and 96 hr, cell viability decreased concentration-dependent. After 24 hr, IC50 concentration of apoptotic spirulina extract significantly increased nitric oxide production in both cell lines ( $p < 0.001$ ). It caused a significant increase in the expression of *ASK1* and *p38* and *Fas* and *FasL* genes in both lines ( $p < 0.001$ ).

**Conclusion:** Spirulina has cytotoxic effects on breast and prostate cancer cells and induces apoptosis by inhibiting nitric oxide production and activation of the Fas/FasL-ASK1-p38 pathway.

**Keywords:** *Spirulina*, Prostate cancer, Breast cancer, Apoptosis.

#### P-95

### Investigating the effects of spirulina on proliferation, apoptosis, nitric oxide release and the expression of Ras/mitogen activated protein kinase pathway genes in cervical cancer cells (CC1-PI 19)

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**Background:** It is well known that the regular consumption of some natural substances, chemopreventive agents, can reduce the risk of certain cancers. Therefore, spirulina an attractive chemotherapeutic agent due to its beneficial health effects. So, identifying treatment methods with fewer side effects is needed.

**Objective:** This study aimed to investigate spirulina's effects on proliferation, apoptosis, nitric oxide secretion and gene expression.

**Materials and Methods:** In this experimental study, after the treatment of cells with spirulina, survival was measured by the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay. The diphenylamine test and Burley's nitric oxide test were used to measure apoptosis using the Griess reaction. Gene expression was measured and quantified by real-time polymerase chain reaction. Ras/mitogen activated protein kinase pathway was performed in cervical cancer cells.

**Results:** The proliferation inhibition and cytotoxic effects of spirulina on cancer cells and fibroblasts were concentration and time dependent. The IC50 of spirulina for cancer cells was  $14.39 \pm 0.87$ ,  $5.19 \pm 0.34$ ,  $1.55 \pm 0.08$ , and  $0.83 \pm 0.008$  for 24, 48, 72, and 96 hr, respectively. Also, IC50 for fibroblast cells was  $14.23 \pm 307.26$ ,  $164.07 \pm 5.11$ ,  $113.56 \pm 1.53$ , and  $85.37 \pm 0.77$  for 24, 48, 72, and 96 hr, respectively. Treatment with the IC50 concentration of spirulina induced a significant increase in apoptosis, and increased the expression of rat sarcoma family genes (*Harvey RAS*, *Kirsten RAS*, and *neuroblastoma RAS*), rapidly accelerated fibrosarcoma (*ARAF*, *BRAF*, and *CRAF*), mitogen-activated protein kinase kinase (*MEK1*, *MEK2*, *MEK3*, *MEK4* and *MEK5*) and inhibited the extracellular signal-regulated kinase gene ( $p < 0.001$ ). Also, the IC50 concentration of spirulina significantly reduced nitric oxide production in cervical cancer cells ( $p < 0.001$ ).

**Conclusion:** Spirulina may have anticancer effects through stimulation of apoptosis and inhibition of the Ras/MAPK signaling pathway.

**Keywords:** *Spirulina*, Cervical cancer, Survival, Apoptosis, Nitric oxide.

#### P-96

### Investigating the effects of spirulina on *Sortilin I* gene expression and response to chemotherapy in ovarian cancer cells

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**Background:** Ovarian cancer with 40% recovery rate, is the most common reason of death of cancerous women in the United States. It is clear that routine intake of certain natural substances, chemopreventive agents, can decrease the incidence of certain cancers. So, spirulina is an attractive chemotherapeutic agent regard to its useful health properties.

**Objective:** This study was conducted to determine spirulina's effects on *Sortilin 1* gene expression and response to chemotherapy of ovarian cancer cells.

**Materials and Methods:** In this experimental study, A2780s cell lines were treated with different concentrations of spirulina (50, 25, 12.5 mg/mL) that solved 70% methanol and were incubated for 24, 48, 72 and 96 hr. Cell viability was checked using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide method. Toxicity was measured by lactate dehydrogenase test. Apoptosis was evaluated by a diphenylamine test. *Sortilin 1* gene expression was measured by real-time polymerase chain reaction. Finally, the effect of simultaneous treatment with spirulina and carboplatin on cell viability was measured. All analysis were done for every spirulina concentration and every time.

**Results:** Cell viability decreased depending on concentration and time after treatment with spirulina. The half-maximal inhibitory concentration of the extract was 17.22, 5.43, 2.80 and 1.17 µg/ml for 24, 48, 72 and 96 hr ( $p < 0.001$ ). Apoptosis increased in a concentration-dependent manner ( $p < 0.001$ ). *Sortilin 1* gene expression in cells decreased in a concentration-dependent manner after 24 hr ( $p < 0.001$ ). After 24 hr of treatment with carboplatin, a decrease in cell viability was observed ( $p < 0.001$ ). The half-maximal inhibitory concentration value of the extract was 28.01 µM for 24 hr. Simultaneous treatment with carboplatin and spirulina extract had a synergistic effect on cell viability ( $p < 0.001$ ).

**Conclusion:** Spirulina hydroalcoholic extract had cytotoxic and apoptosis-inducing effects on ovarian cancer cells and increased the sensitivity of these cells to carboplatin chemotherapy agent.

**Keywords:** *Spirulina*, *Sortilin 1* gene, *Chemotherapy*, *Ovarian cancer*, *Carboplatin*.

## P-97

### Nicotine alters sperm *miR-151-5p* and expression of *Cep72* in testicular tissue

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**Background:** Nicotine as a toxic agent of cigarette smoke has adverse effects both on germ and somatic cells in the testis. Overexpression or knockout of the

centrosomal protein 72 (*Cep72*) gene in somatic cells has been shown to impair the mitotic process. *Cep72* is specifically expressed in spermatocytes and round spermatids in testicular tissue, indicating its importance in male germ cell development. In addition, microRNA-151-5p (*miR-151-5p*) has emerged as a critical post-transcriptional regulator that may modulate the expression of genes associated with cellular stress and proliferation.

**Objective:** This study investigated the effects of nicotine exposure on the expression of the *Cep72* in testicular tissue and *miR-151-5p* in mouse sperm.

**Materials and Methods:** In this experimental study 16 adult BALB/C male mice (8-10 wk, 25-30 gr), were divided into two groups (n = 8/each): a control group receiving saline and an experimental group received 0.6 mg/kg nicotine intraperitoneally. Treatment lasted for 35 days. The numbers of different types of germ cells, Sertoli and Leydig cells were counted in the testis in all stages of spermatogenesis (early, mid, and late) using histological and image analyzer methods. The quantitative real-time-polymerase chain reaction method was used to assess the expression levels of *Cep72* and *miR-151-5p* in testicular tissue and epididymal sperms, respectively.

**Results:** Nicotine induced a significant decrease in Leydig cells ( $p = 0.001$ ) without having a change in Sertoli cells. All types of germ cells were mostly affected by nicotine in different stages of spermatogenesis. A significant decrease in *Cep72* gene expression was observed in the testicular tissue of the nicotine group compared to the control group ( $p = 0.028$ ). A significant increase in *miR-151-5p* expression was observed in the sperm of the nicotine group compared to the control group ( $p = 0.003$ ).

**Conclusion:** These findings suggest that nicotine may disrupt normal testicular germ and somatic cell development and function by modulating key regulatory pathways involving *Cep72* and *miR-151-5p*. The roles of *Cep72* and *miR-151-5p* in this process are particularly significant, offering potential targets for future research and therapeutic advancements.

**Keywords:** *Cep72*, *Nicotine*, *MicroRNA-151*, *Testis*.

## P-98

### Investigating the role of DNA repair-related factors in non-obstructive azoospermia: A bioinformatic study

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**Background:** Non-obstructive azoospermia (NOA) represents the most severe type of male infertility, affecting about 1% of men worldwide.

**Objective:** This study aimed to identify differentially expressed genes (*DEGs*) between NOA samples and normozoospermia, as well as to investigate how DNA repair related factors influence the observed changes in gene expression.

**Materials and Methods:** The DEGs analysis utilized data from the GSE190752 datasets found in the Gene Expression Omnibus. These DEGs were compared with a list of known human DNA repair genes sourced from the REPAIRtoire database to identify overlaps, termed REP-DEs. Pathways enrichment analysis associated with REP-DEs was performed using the ToppGene dataset. Additionally, the protein-protein interaction (PPI) network, co-expression modules, and identification of hub genes for REP-DEs were examined using the STRING database and Cytoscape software to elucidate the regulatory mechanisms at play.

**Results:** The analysis of the GSE190752 dataset, conducted using the EdgeR package, compared the NOA groups with the control tissue group and identified 7,716 DEGs. DEGs were then cross-referenced with a list of 154 DNA repair-related genes, resulting in the identification of 45 REP-DEs. The ToppGene database facilitated the identification of enriched KEGG pathways linked to the REP-DEs, applying a p-adjusted value threshold of less than 0.01 to establish statistical significance. The pathways identified included base excision repair, nucleotide excision repair, homologous recombination, DNA replication, and mismatch repair. Following the construction of the PPI network, three co-expression clusters were identified using the MCODE and CytoHubba applications. The top 5 hub genes in the PPI network, as determined by CytoHubba, were *EXO1*, *RAD51*, *BRCA1*, *MSH2*, and *RAD51C*.

**Conclusion:** This expression study highlighted the significance of DNA repair-related factors in NOA.

**Keywords:** Infertility, Male, RNA-seq, Protein interaction maps, DNA repair.

## P-99

### Investigating the role of L-carnitine in reducing reactive oxygen species and modulating apoptotic gene expression in mouse oocytes exposed to cyclophosphamide

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**Background:** Cyclophosphamide (CP) is a commonly used chemotherapeutic drug recognized for its adverse effects on fertility in women. Conversely, L-carnitine (LC), an antioxidant, has demonstrated promising protective properties against infertility.

**Objective:** The purpose of this study was to investigate the impact of CP and LC on reactive oxygen species (ROS) levels and the expression of apoptotic genes in female mice.

**Materials and Methods:** In this experimental study, 24 NMRI female mice (aged 6-8 wk, weighing 30 ± 5 grams) were randomly divided into 4 groups (n = 6 /each): control group: received normal saline via intraperitoneal (IP) injection for 10 days. CP group: received a single IP injection of CP (75 mg/kg) on the 10<sup>th</sup> day of the experiment. LC group: treated with LC (200 mg/kg) via IP injection for 10 days. LC+CP group: received LC for 10 days, followed by a single CP injection (IP) on the 10<sup>th</sup> day. Following the 10-day treatment period, superovulation was induced in all groups, the oviducts were excised, and oocytes were harvested. The expression levels of apoptotic genes - Bcl2-associated X (*Bax*), *Caspase-3*, and B-cell lymphoma 2 (*Bcl2*)- were analyzed using real-time polymerase chain reaction. Additionally, intracellular ROS levels were assessed using dichloro-dihydro-fluorescein diacetate fluorescence staining.

**Results:** The administration of LC in the LC+CP group resulted in a significant increase in *Bcl2* gene expression (p = 0.012) while simultaneously decreasing the levels of *Bax* and *Caspase-3* compared to the CP group (p = 0.034 and p = 0.040, respectively). Moreover, LC treatment significantly lowered ROS levels in the LC+CP group in comparison to the CP group (p < 0.001).

**Conclusion:** The results of this study indicate that LC effectively reduces CP-induced ROS levels and influences the apoptotic pathway in mouse oocytes by decreasing the expression of *Bax* and *Caspase-3*, while enhancing *Bcl2* expression. These findings highlight the potential of L-carnitine as a therapeutic agent for protecting against infertility induced by chemotherapy.

**Keywords:** L-carnitine, Cyclophosphamide, Apoptosis, Gene expression, Reactive oxygen species.

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## P-100

### Molecular mechanisms of azoospermia through reanalysis of RNA-seq results: A bioinformatic study

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**Background:** Azoospermia, the absence of sperm in semen, is a major cause of male infertility, necessitating a deeper understanding of its underlying molecular mechanisms.

**Objective:** The goal is to identify differentially expressed genes and their associated biological pathways through advanced bioinformatics tools.

**Materials and Methods:** In this in silico study, RNA sequencing (RNA-seq) data were processed using Cytoscape to visualize gene interaction networks and perform gene ontology (GO) enrichment analysis. This study reanalyzes RNA-seq data from 3 gene expression omnibus datasets: GSE51111, GSE45885, and GSE100019. Inclusion criteria for the datasets were studies focused on azoospermia with available RNA-seq expression data and clear differential expression of genes. Exclusion criteria included studies lacking relevant clinical data, those not published in English, and datasets with insufficient quality of sequencing data. This analysis categorized differentially expressed genes into relevant biological processes, molecular functions, and cellular components to uncover significant pathways implicated in azoospermia.

**Results:** Our findings highlight key biological processes related to azoospermia, supported by significant evidence: nucleoplasm enrichment (GO:0005654): many differentially expressed genes localize in the nucleoplasm ( $p = 4.79E-21$ ), indicating potential disruptions in nuclear function affecting sperm production (e.g., late translation, ribosomal protein L5, heterogeneous nuclear ribonucleoprotein U, parkinsonism associated protein 7). Nucleic acid binding (GO:0003676): genes involved in nucleic acid binding ( $p = 6.46E-21$ ) suggest altered RNA interactions contribute to azoospermia (notable genes: ribosomal protein L30, dicer 1, Ribonuclease III). RNA Metabolism: significant enrichment in RNA metabolism pathways ( $p = 1.28E-20$ ) indicates disruptions in RNA processes (key genes: ribosomal protein L9, 5'→3' exoribonuclease 1). Translation processes: translation-related genes ( $p = 6.81E-19$ ) are important for sperm development (e.g., ribosomal protein 14). Peptide biosynthesis: significant involvement in peptide biosynthesis ( $p = 1.70E-19$ ), indicating disrupted peptide formation (e.g., *RPL5*). These results show azoospermia is associated with major abnormalities in critical biological pathways.

**Conclusion:** This RNA-seq analysis reveals important pathways in azoospermia, laying the groundwork for future research into male infertility and potential therapies.

**Keywords:** Azoospermia, RNA-seq, Gene ontology, Nucleoplasm, Nucleic acid binding, Peptide biosynthesis.

## P-101

### Identification of transcriptome changes in polycystic ovarian syndrom using bioinformatics analysis

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**Background:** MicroRNAs (miRNAs) are approximately 22-nucleotide long small RNA molecules that modulate gene expression by binding either partially or fully to their complementary sequences within mRNAs or their promoters.

**Objective:** This study focused on the gene expression profiles of cumulus cells in patients diagnosed with polycystic ovary syndrome (PCOS), utilizing data from the gene expression omnibus (GEO) database.

**Materials and Methods:** In this in silico study, we analyzed the standardized gene expression microarray data obtained from the GEO database, specifically using the dataset GSE277906:GPL24676 from the Illumina Novaseq6000 platform. This dataset encompasses gene expression comparisons between PCOS patients and control individuals. Data analysis was conducted using R software (version 4.4.2), employing the limma package to identify significant differential gene expressions. A strict criterion ( $p < 0.05$ ) and log fold change were applied, resulting in the identification of key genes demonstrating notable differences in expression.

**Results:** Our analysis revealed that the nicastrin (*NCSTN*), cytochrome oxidase assembly factor (*COX19*), and mitochondrially encoded NADH dehydrogenase1 (*MTND1*) genes exhibited decreased expression in PCOS women. In contrast, the expression of *OFD1* centriole and centriolar satellite protein, phosphoenolpyruvate carboxykinase1 (*PCK1*), syndecan-2 (*SDC2*), splicing factor serine-rich5 (*SRSF5*), polypyrimidine tract binding protein 2 (*PTBP2*), voltage dependent anion channel 2 (*VDAC2*), and zinc finger Imprinted 3 (*ZIM3*) were increased. The miRNAs identified in our study target these genes and are involved in pathophysiology of PCOS. The specific miRNAs identified include miR-1-3p, miR-548n, miR-619-5p, miR-92a, miR-320, miR-let7, miR-17-5p, and miR-20a-5p. We further analyzed the Kyoto encyclopedia of genes and genomes (KEGG) pathways associated with these miRNAs, linking them to key cellular processes in PCOS, including the steroid and foxo orthologs from arthropods (*FOXO*) signaling pathways. Additionally, we examined protein-protein interactions among the downregulated genes, highlighting the interactions between *COX19* and *MTND1* with cytochrome oxidase assembly factor 6 (*COA6*), translocase of inner mitochondrial membrana22 (*TIMM22*), and cytochrome Oxidase Assembly Factor 1 (*COA1*). subunit (*PSENEN*), and rhomboid like2 (*RHBDL2*).

**Conclusion:** These findings improve our understanding of the molecular mechanisms involved and offer

potential for developing innovative diagnostic and therapeutic strategies for managing the disorder.

**Keywords:** PCOS, Transcriptome, Microarray, Bioinformatics analysis, GEO database.

## P-102

### Evaluation of the leukaemia inhibitory factor and leukaemia inhibitory factor receptor genes expression affecting endometrial receptivity in the endometrium of women with secondary infertility due to isthmocele compared to fertile group

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**Background:** Secondary infertility is an issue affecting approximately 5% of the population and refers to women who have already had a baby in the past without any fertility treatments. Isthmocele or niche is thought to cause secondary infertility, resulting from a previous caesarean section, and may affect embryo implantation due to the stagnation of menstrual blood in the niche. Leukaemia inhibitory factor (*LIF*) and its receptor (*LIFR*) are mainly produced by the maternal endometrium at the time of implantation. It has been reported that disruption in the expression of *LIF* and *LIFR* can cause embryo implantation failure.

**Objective:** The purpose was to investigate the effect of isthmocele on the expression level of *LIF* and *LIFR* genes during the window of implantation.

**Materials and Methodes:** This ethically approved case-control study at the Royan Institute, Tehran, Iran involved endometrial tissue sampling between 2023-2024, including 10 infertile women with isthmocele grade 3 (case group) and 10 fertile women (control group). Women in both study groups were < 40 yr old, with normal ovarian response and reserve. The isthmocele grade 3 group had a history of cesarean, confirmed via sonohysterography. The control group included women with at least one natural birth who visited the Royan Institute, Tehran, Iran for family planning. Endometrial biopsy during the window of implantation was done for both groups. *LIF* and *LIFR* mRNA gene expression were performed using quantitative polymerase chain reaction.

**Results:** Using quantitative polymerase chain reaction, it was demonstrated that the expression levels of both *LIF* and *LIFR* genes were significantly higher ( $p \leq 0.050$ ) in the isthmocele group compared to the control group.

**Conclusion:** The uterine incision during a cesarean section is generally made transversely in the lower

uterine segment and could lead to the accumulation of mucus and blood in the defect. The presence of blood and mucus in the niche may cause impaired embryo implantation through the change of some crucial gene expression, the same as *LIF* and *LIFR*. Given the role of both the *LIF* gene and its receptor in enhancing endometrial receptivity, the findings of this study suggest that excessive overexpression of these genes may hinder embryo implantation.

**Keywords:** Endometrium, Infertility, *LIF*, *LIFR*.

## P-103

### Relationship between oocyte maturation and *TSC1* and *miR-92b-3p* genes expression in cumulus cells of patients with polycystic ovary syndrome

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**Background:** Polycystic ovary syndrome (PCOS) is a multifactorial disease caused by an endocrine disorder. One of the symptoms of PCOS is hyperandrogenism, which can disrupt follicular development. This disorder can cause problems in oocyte development and embryonic development. Cumulus cells surround the oocyte and have a reciprocal functional relationship with the oocyte. Therefore, gene expression in cumulus cells is associated with oocyte development and maturation. The PI3K/PTEN/Akt and TSC/mTOR signaling pathways play a crucial role in regulating ovarian function through the activation and survival of primordial follicles, division and differentiation of granulosa cells and meiotic maturation of oocytes. Dysregulation of these signaling pathways leads to infertility. *TSC1*, an essential component of the PI3K/AKT/mTOR signaling pathway, plays an important role in cell growth, proliferation, and survival processes. Studies have shown that miRNAs are present in various body fluids, including the follicular fluid of women with PCOS. The *miR-92b-3p* is involved in transcriptional regulation in the accumulation of primordial follicles and is a novel therapeutic target in the diagnosis and treatment of PCOS.

**Objective:** The aim of this study was to investigate the expression levels of *TSC1* and *miR-92b-3p* genes in cumulus cells of GV oocytes compared to cumulus cells of metaphase II oocytes in women with PCOS.

**Materials and Methods:** In this case-control study, the case group consisted of cumulus cells from GV oocytes and the control group consisted of cumulus cells from MII oocytes, which were collected by embryologist from 25 women with PCOS who referred to the Yazd

Reproductive Sciences Institute, Yazd, Iran for infertility treatment. The inclusion criteria for the study were age 25-40 yr, infertility due to PCOS, and the presence of more than 10 oocytes in the follicular fluid after oocyte retrieval. The samples were stored in a -70°C freezer until RNA extraction. Total RNA was extracted from cumulus cells and cDNA was synthesized. The cDNA was used as a template for real-time polymerase chain reaction. To evaluate changes in *TSC1* and *miR-92b-3p* genes expression, the relative quantification method ( $\Delta$ Ct) was compared between the groups.

**Results:** According to our studies, the relative expression of *TSC1* gene in cumulus cells of GV oocytes was significantly increased compared to cumulus cells of MII oocytes in patients with PCOS ( $p = 0.0007$ ). Subsequently, the relative expression of *miR-92b-3p* in cumulus cells of GV oocytes was significantly decreased compared to cumulus cells of MII oocytes in patients with PCOS ( $p = 0.0141$ ).

**Conclusion:** Cumulus cell gene expression analysis offers a non-invasive method to study oocyte status. This study suggests that *TSC1* and *miR-92b-3p* may play a role in oocyte quality in women with PCOS. Larger cohorts are recommended for confirmation and further information. Further studies on the expression of other genes that may play a role in oocyte quality are also recommended.

**Keywords:** Oocyte maturation, Cumulus cells, Polycystic ovary syndrome, *TSC1*, *miR-92b-3p*.

#### P-104

### Investigating the effect of altered miR-185-5P expression on ovarian response in women with diminished ovarian reserve: A case-control study

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**Background:** Diminished ovarian reserve (DOR) is defined as a condition that affects fertility by reducing the reproductive potential of the ovary. The factor that can negatively affect the quality and quantity of oocytes in the ovary is the alteration of the expression profile of cumulus cells (CCs). Based on the research, circulating miRNAs play an important role in ovarian function and can potentially serve as important biomarkers for predicting ovarian function.

**Objective:** To investigate the expression of miR-185-5P in serum and CCs in order to find a diagnostic biomarker for DOR patients.

**Materials and Methods:** In this case-control study, the participants included 20 women with DOR and 20 women with a normal ovarian reserve between 20-34 yr of age referred to Yazd Reproductive Sciences Institute, Yazd, Iran. Serum and CC were collected and real-time polymerase chain reaction was performed to examine the expression level of miR-185-5P.

**Results:** Our results showed that the increased expression of miR-185-5P was significantly higher in both serum and CCs of women with DOR compared to the control group ( $p = 0.0019$  and  $p = 0.0008$ , respectively).

**Conclusion:** The present study shows that examining this miRNA can be a promising diagnostic biomarker for predicting ovarian reserve and its response to stimulation protocols.

**Keywords:** miR-185-5P, Diminished ovarian reserve, Ovarian response, Cumulus cells.

#### P-105

### Myo-inositol improves spermatogenesis in diabetic rats by reducing inflammation and oxidative stress through the *Bax/Bcl2/Nrf2* pathway

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**Background:** Type 2 diabetes mellitus causes impaired spermatogenesis by increasing inflammation and apoptosis and decreasing testosterone synthesis in testicular tissue. Myo-inositol is a six-carbon sugar alcohol and a natural antioxidant. The concentration of free myo-inositol in the testes is 30-40 times higher than its concentration in the blood. Given that myo-inositol synthesis is reduced by 50% in the testes of diabetic individuals and is also excreted in the urine.

**Objective:** The present study aim to investigate the effect of myo-inositol on spermatogenesis in diabetic rats by evaluating inflammatory factors, oxidative stress, expression of apoptosis-regulating genes, and terminal deoxynucleotidyl transferase dUTP nick end labeling test.

**Materials and Methods:** Eighteen adult male Wistar rats were divided into 3 groups: control, diabetic (streptozotocin [65 mg/kg] + nicotinamide [110 mg/kg]) and diabetic + myo-inositol (300 mg/kg). The treatment period with myo-inositol was 56 days (by gavage). After the treatment period and dissection of the rats, each pair of testes was removed for histological, molecular, and biochemical examination. Testicular supernatant was used to evaluate the expression of *Bax*, *Bcl2*, and *Nrf2* genes (using real-time polymerase chain reaction), inflammatory factors and testosterone levels (using enzyme-linked immunosorbent assay kits), and oxidative stress factors including superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) (using spectrophotometer). Also, tissue sections prepared from the testis were used

for histopathological examination of the testis (counting the number of spermatogenic, Leydig, and Sertoli cells) using the stereological method as well as the terminal deoxynucleotidyl transferase dUTP nick end labeling test.

**Results:** In the diabetic group, there was a notable elevation in the average levels of IL-6, TNF- $\alpha$ , *Bax*, and MDA ( $p \leq 0.001$  for all). Additionally, the number of apoptotic cells increased significantly ( $p \leq 0.001$ ), while the mean concentrations of TAC, SOD, *Nrf2*, *Bcl2*, testicular testosterone ( $p \leq 0.001$  for all), as well as the numbers of spermatogonia, spermatocytes, spermatids, Sertoli cells, and Leydig cells all decreased markedly ( $p = 0.000$  for each comparison), when compared to the control group. Conversely, in the diabetic + myo-inositol group, there was a significant reduction in the levels of IL-6 ( $p = 0.021$ ), TNF- $\alpha$  ( $p \leq 0.001$ ), *Bax* ( $p = 0.019$ ), MDA ( $p \leq 0.001$ ), and the number of apoptotic cells ( $p \leq 0.001$ ). At the same time, this group showed a substantial rise in antioxidant levels such as TAC ( $p = 0.024$ ), SOD ( $p = 0.013$ ), *Nrf2* ( $p \leq 0.001$ ), and *Bcl2* ( $p = 0.044$ ), along with increased testicular testosterone and a higher count of spermatogonia, spermatocytes, spermatids, Sertoli cells, and Leydig cells ( $p \leq 0.001$  for all), compared to the diabetic group.

**Conclusion:** The results of our study showed that myo-inositol supplementation reduces apoptosis in the testis by reducing oxidative stress and inflammation caused by type 2 diabetes, leading to an increase in the number of spermatogenic and somatic testicular cells and reducing the destructive effects of type 2 diabetes on the process of spermatogenesis.

**Keywords:** Type 2 diabetes, Spermatogenesis, Myo-inositol, Apoptosis, Inflammation.

## P-106

### Upregulation of Long non-coding RNA-SRLR and Interleukin-1 $\beta$ in women with polycystic ovary syndrome

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**Background:** Polycystic ovary syndrome (PCOS) affects 70% of women with ovulation difficulties. The pathogenesis is not fully understood and is largely thought to involve a complex and unknown interaction between environmental and genetic factors. The correct diagnosis of PCOS is still difficult despite the Rotterdam criteria. Oocyte maturation depends on the interaction between it and follicular cells, including cumulus and granulosa cells. Studying granulosa cell proliferation, differentiation, and transformation is crucial for understanding folliculogenesis. Differences in long non-coding RNAs (*lncRNAs*) and pro-inflammatory

cytokines such as Interleukin-1 $\beta$  (*IL-1 $\beta$* ) expression are linked to gynecological disorders.

**Objective:** This study aimed to investigate the expression of *LncRNA-SRLR* and the *IL-1 $\beta$*  genes in women with PCOS compared to non-PCOS women.

**Materials and Methods:** In this case-control study, samples of granulosa cells isolated from the surrounding oocytes of 28 PCOS and 18 non-PCOS women who referred to the IVF center of Beheshti hospital, Kashan, Iran between 2022-2023, were studied. These women had visited the infertility center for in vitro fertilization or Intra-cytoplasmic sperm injection on the day of oocyte retrieval. Following RNA extraction and c-DNA synthesis, the expression levels of the *SRLR* and *IL-1 $\beta$*  genes in the granulosa cells were assessed using quantitative polymerase chain reaction.

**Results:** The expression levels of *SRLR* ( $p \leq 0.001$ ) and *IL-1 $\beta$*  ( $p = 0.038$ ) genes were significantly higher in the granulosa cells of women with PCOS compared to non-PCOS women.

**Conclusion:** Elevated expression of the *LncRNA-SRLR* and *IL-1 $\beta$*  genes in granulosa cells leads to the pathophysiological processes of PCOS and increased inflammation.

**Keywords:** Long non-coding RNA, PCOS, Granulosa cell, Interleukin-1beta.

## P-107

### Fluoxetine reduces the quality of vital parameters of normal human sperm by affecting the expression of genes related to apoptosis: A laboratory study

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**Background:** Depression, a prevalent mental health condition, impacts countless individuals annually. Fluoxetine, a widely used antidepressant, is valued for its safety and effectiveness. As a selective serotonin reuptake inhibitor, fluoxetine enhances serotonin levels in the brain. However, research suggests that fluoxetine may have detrimental effects on certain cell types, including ovarian epithelial and hippocampal cells, by potentially increasing oxidative stress and contributing to reduced cell survival.

**Objective:** Since many men of reproductive age use fluoxetine to treat their depression, it is essential to investigate its effect on sperm quality. The aim of this study was to evaluate the effect of fluoxetine on motility, viability, DNA fragmentation, and expression of genes related to apoptosis of human sperm in vitro.

**Materials and Methods:** The type of this study was experimental. Semen samples were collected from 30 fertile men and divided into 2 groups including: control (no treatment, 1-hr incubation), and fluoxetine-treated

(5  $\mu$ M fluoxetine, 1-hr incubation). The study evaluated sperm motility (total, progressive, and non-progressive), viability, reactive oxygen species (ROS) levels, and the expression of apoptosis-related genes (*BAX*, *BCL2* and *CASPASE3*). Data were analyzed using repeated measures analysis.

**Results:** In the fluoxetine group, the mean percentage of total motility, progressive motility, viability, and expression level of the antiapoptotic gene *BCL2* showed a significant decrease compared to the control group ( $p < 0.001$ ), while the mean percentage of non-progressive motility, DNA fragmentation, ROS level, and expression levels of the apoptotic genes *BAX* and *CASPASE3* showed a significant increase in the fluoxetine group compared to the control group ( $p < 0.001$ ).

**Conclusion:** Our results indicate that fluoxetine negatively impacts human sperm quality in vitro by reducing motility, viability, and anti-apoptotic gene expression, while increasing DNA fragmentation, ROS levels, and apoptotic gene expression. These findings suggest oxidative stress and apoptosis as key mechanisms of damage, raising concerns about its potential effects on male reproductive health.

**Keywords:** Fluoxetine, Sperm, Apoptotic genes.

## **P-108 Artificial intelligence in preventing reproductive failure and genetic insights**

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Artificial intelligence (AI) is transforming reproductive medicine by enhancing the analysis of genetic factors associated with infertility. AI tools facilitate accurate diagnoses and personalized strategies by identifying causal genetic variants through advanced genomic and epigenomic analysis. This study highlights AI's transformative role in reproductive medicine, emphasizing genetic insights. The goal of this study was to evaluate the transformative role of AI in reproductive medicine by examining how AI tools enhance the analysis of genetic factors associated with infertility, thereby facilitating accurate diagnoses and personalized treatment strategies. A comprehensive literature review was conducted between 2018-2024 using databases such as PubMed, Nature, and MDPI with the keywords "Artificial Intelligence", "infertility", "genetic variants", "personalized treatment", "IVF", and "machine learning". Genomic analysis tools including Emedgene, AI-MARRVEL, DNAnexus, Rosetta, Bionano's VIA, DeepMetabolism, Basepair, insitro, Tinybio and PetaGene were evaluated for their roles in medical and reproductive genetics. Additionally, AI-based embryo

selection systems such as IVF Australia's technology and the STORK framework were assessed for their effectiveness in improving reproductive outcomes. AI-powered genomic analysis tools have significantly advanced the diagnosis and management of infertility by improving the accuracy and efficiency of genetic variant interpretation. For example, Emedgene automates variant classification using machine learning algorithms like Random Forest and Support Vector Machines, reportedly achieving an accuracy of up to 95% in identifying disease-causing mutations compared to traditional methods. AI-MARRVEL prioritizes candidate gene variants by integrating clinical and genomic data with a scoring system based on Bayesian networks, enhancing the identification of causative mutations. Platforms like DNAnexus facilitate comprehensive genomic data management, enabling seamless integration of multi-omics data for better clinical insights; DNAnexus uses various algorithms for data processing and integration but the accuracy varies depending on the specific application. Rosetta aids in understanding protein structural impacts of mutations, crucial for interpreting variant pathogenicity; Rosetta employs computational modeling and energy function optimization to predict protein structures, but accuracy depends on protein complexity. Bionano's VIA integrates diverse genomic data to detect structural variants often missed by conventional methods using proprietary algorithms to increase accuracy. DeepMetabolism predicts phenotypic consequences of genetic changes, informing personalized treatment strategies through machine learning models trained on large-scale metabolomic data. Tools such as Basepair streamline next-generation sequencing analysis, accelerating the discovery of infertility-associated markers. Insitro leverages machine learning for drug discovery targeting genetic causes of reproductive disorders. Tinybio explores the microbiome's influence on reproductive health through metagenomic analysis. Lastly, PetaGene ensures secure and efficient genomic data storage and access, facilitating large-scale genetic studies. Collectively, these AI-driven platforms contribute to more precise genetic diagnoses, improved embryo selection, and personalized therapeutic approaches, ultimately enhancing reproductive outcomes. AI integration in reproductive medicine improves diagnostic accuracy and treatment efficacy. From tools like DeepVariant to platforms like DNAnexus, AI is reshaping infertility research and treatment. Address ethical considerations, particularly data privacy and informed consent.

**Keywords:** Artificial intelligence, Reproductive genetics, Infertility, Genomic analysis.

## **P-109 The prevalence of human papillomavirus genotypes among women referred to the Yazd Reproductive Sciences Institute, Yazd, Iran from 2022-2024**

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**Background:** Human papillomavirus (HPV) causes most genital warts and anogenital cancers, including cervical cancer in women. HPV primarily spreads sexually and damages epithelial cells. Over 200 genotypes have been found to date. These genotypes are classified into high-risk and low-risk due to their carcinogenic potential. HPV-6 and HPV-11 are linked to benign conditions like genital warts and low-grade cellular changes with limited tumorigenic potential, while HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 strongly link to cancer development. Cervical cancer is the second leading cause of cancer deaths in women due to recurrent HPV-16 and HPV-18 infections. Molecular HPV genotype detection complements Pap smears, colposcopy, and biopsy for precise diagnosis and treatment.

**Objective:** This cross-sectional study aimed to investigate the prevalence of HPV genotypes of 300 women referred by gynecologists to the molecular diagnostics for reproductive infectious disorders at the Yazd Reproductive Sciences Institute, Yazd, Iran between 2022 and 2024.

**Materials and Methods:** The Pap smear samples were collected from the anogenital regions of women during the study period (2022-2024). HPV DNA was extracted, and genotyping was performed using a real-time polymerase chain reaction assay. To assess the relationship between HPV infection and age, participants were categorized into 5 age groups ( $\leq 20$ , 20-30, 30-40, 40-50, and  $\geq 50$  yr).

**Results:** A total of 16 HPV genotypes, including both high-risk and low-risk variants, were identified. Among the 300 participants, 23.33% were infected with at least one HPV genotype. The most prevalent genotypes included high-risk HPV-16 (5.66%), HPV-51, 56, and 66 (3.66%), and HPV-31, 35, 39, and 68 (3.33%). Low-risk genotypes HPV-6 and HPV-11 were detected in 5.33% of cases. Co-infections involving  $\geq 2$  genotypes were observed in 4% of participants, with the most frequent co-infection involving HPV-16 and HPV-6/11 (33.3%). The highest prevalence of HPV infection was observed in women aged 30-40 yr (48.57%), peaking between 36 and 40 yr. However, no statistically significant correlation was found between age and HPV infection ( $p = 0.45$ ).

**Conclusion:** The reported prevalence of HPV genotypes in this study was relatively high compared to global statistics, likely due to the referral of most participants by gynecologists rather than through routine screening programs. Given that cervical cancer is predominantly caused by persistent infections with high-risk HPV genotypes, implementing comprehensive screening strategies, particularly those focusing on early detection of high-risk genotypes, is crucial for the

prevention and targeted treatment of gynecological cancers. This study also provides valuable epidemiological insights into HPV genotype distribution, which could inform future vaccination policies and public health strategies.

**Keywords:** Human papillomavirus Cervical cancer, Genotyping, Prevalence, Yazd.

## **P-110** **Obesity alters ovarian insulin-like growth factor-1 expression in rats**

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**Background:** Ovarian insulin-like growth factor 1 (*IGF-1*) plays a critical role in reproductive health and metabolic regulation. It influences follicular development, steroidogenesis, and overall ovarian function. In obese individuals, the dysregulation of *IGF-1* signaling in the ovaries can have profound implications for fertility and metabolic homeostasis.

**Objective:** The aim of this study is the evaluation of the expression of *IGF-1* in the ovary in obese rats induced by high-fat diet (HFD).

**Materials and Methods:** For 16 wk, 6 wk-old Wistar rats had the option to receive either a standard diet (10% fat,  $n = 12$ ) or a high fat diet (HFD; 60% fat,  $n = 12$ ) as their diet. Biochemical and molecular tests were utilized to evaluate ovary function and expression of *IGF-1*, including enzyme-linked immunosorbent assay and quantitative polymerase chain reaction.

**Results:** Rats fed an HFD showed a significant increase ( $p < 0.050$ ) in Lee index, insulin resistance, liver enzymes, glucose, and triglycerides compared with those on a standard diet. Furthermore, rats on an HFD showed alterations in folliculogenesis ( $p < 0.050$ ) and an increase in expression of ovarian *IGF-1* ( $p < 0.050$ ).

**Conclusion:** Obesity disturbs local *IGF-1* signaling in ovary tissue and may affect follicles development.

**Keywords:** Obesity, Ovary, *IGF-1*, Folliculogenesis.

## **P-111** **The effect of exenatide, on adiponectin system expression in polycystic ovary syndrome**

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**Background:** Polycystic ovary syndrome (PCOS) is a common multifaceted and intricate endocrine disorder

that affects 5-10% of women of reproductive age. Serum levels of adiponectin, which is a peptide hormone, released from fat cells that control insulin sensitivity, lipid metabolism, and glucose levels through its anti-fibrotic and antioxidant properties, are low in PCOS women.

**Objective:** This study aimed to investigate the effects of exenatide on mRNA expression levels of the adiponectin system in PCOS rats.

**Materials and Methods:** In our cross-sectional study, 28 normal cyclicity female Wistar rats weighing 175-200 gr were divided into 4 groups (n = 7/each). No medication was given to the normal group. After receiving estradiol valerate to develop PCOS, the PCOS + vehicle group was divided into PCOS + E50 and PCOS + E100 groups, and they were given doses of exenatide of 50 or 100 µg/kg, respectively. The mRNA expression of adiponectin and adiponectin receptor 1 (Adipo-R1) was evaluated using a semi-quantitative real-time polymerase chain reaction.

**Results:** Our study showed that the level of mRNA Adipo-R1 was increased in the PCOS group compared to the normal group (p < 0.05). Both doses of 50 and 100 µg/kg exenatide can decrease the Adipo-R1.

**Conclusion:** Our findings suggested that exenatide may improve rats with PCOS by regulating the molecules of adiponectin and its receptor.

**Keywords:** Polycystic ovary syndrome, Exenatide, Adiponectin.

## P-112

### Annexin A5/M2 haplotype in couples who suffer from recurrent implantation failure

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**Background:** Recurrent implantation failure (RIF) is defined as ≥ 3 failed fresh or frozen embryo transfer (ET) cycles with at least four optimal quality embryos in women < 40 yr old. Although several causes such as uterine abnormalities, parental genetic disorders and endometrial factors may lead to this condition, many RIF cases remain unexplained. Thrombophilias is considered one of the potential causes of RIF. Annexin

A5 (ANXA5) has a potent anticoagulant effect which is expressed on placenta and syncytiotrophoblast cells. The M2 haplotype which are 4 single nucleotide polymorphisms that are inherited together from the parents can decrease the expression of ANXA5 and lead to placental complication. Several studies have shown that variation in the promoter of ANXA5 represents a risk factor for recurrent pregnancy loss but the relation of this genetic variation with RIF has not been studied in Iranian population.

**Objective:** This study purpose is to determine the potential role of the M2/ANXA5 haplotype as a risk factor for unexplained RIF in Iranian population.

**Materials and Methods:** In this ongoing case-control study, 20 infertile couples with history of three or more failed ET cycles without known cause (unexplained RIF group) and 12 couples who had previously natural live births and referred to Royan Institute, Tehran, Iran for sex selection of next child (control group) were enrolled between 2024-2025. In both groups, women's and men's age was < 40 and < 45 yr old, respectively. All couple in RIF group were screened for uterine anatomical anomalies, female hereditary thrombophilia, parental genetic disorders and female endocrine dysfunction. In case of the presence of any mentioned factors, the RIF couple were not included. After taking written informed consent, blood samples were drawn from studied couples (females and males), then DNA was extracted by using the salting out method. The primer of ANXA5 core promotor was designed using primer 2 and BLAST. After polymerase chain reaction on the extracted DNA, each sample was used for Sanger sequencing.

**Results:** Till now, 20 unexplained RIF and twelve fertile couples (control) were enrolled. The mean age of men was 40.16 yr old and average age of women were 35.31 yr old. Mean number of previous failed ET was 4.12 cycle in unexplained RIF group. M2/ANXA5 haplotype was observed only in 2 men of RIF group while no man in control group had this haplotype. This difference between groups was not significant (p = 1.93). None of women in both groups had this haplotype.

**Conclusion:** According to this preliminary data, the M2/ANXA5 haplotype was only found in male partner of unexplained RIF couples. ANXA5 expression is required for the fetal compartment at the fetomaternal interface to facilitate anticoagulation and haplotype M2 of this factor could reduce its expression levels. For a definite conclusion, the completion of the sample size is needed.

**Keywords:** Infertility, Haplotypes, Annexin A5, IVF failure.

## P-113

### Protective effect of hesperidin on malathion-induced ovarian toxicity in mice: The role of miRNAs, inflammation, and apoptosis

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**Background:** Malathion, a widely used organophosphate pesticide, is known for its relatively low toxicity and broad application. However, it has been identified as a female reproductive toxicant, exerting its harmful effects through mechanisms such as oxidative stress, apoptosis, autophagy, and hormonal imbalances. Hesperidin, a flavonoid belonging to the flavanone subclass, possesses multiple biological activities, including antioxidant and anti-inflammatory properties, which may mitigate these toxic effects.

**Objective:** The objective of this study was to evaluate the impact of hesperidin and malathion on the expression of microRNAs (miRNAs) and genes involved in apoptosis and inflammation.

**Materials and Methods:** In this experimental study, 40 female BALB/c mice (n = 40) were randomly assigned into 4 groups: hesperidin (20 mg/kg), malathion (3 mg/kg), hesperidin + malathion, and control. Following 35 days of intraperitoneal treatment, the mice were euthanized. The left ovaries were used for the analysis of miRNA-146a-5p, miRNA-129-3p, miRNA-96-5p, nuclear factor kappa B (*NF-κB*), B-cell lymphoma 2-associated X protein (*Bax*), and B-cell lymphoma 2 (*Bcl-2*) gene expression via real-time quantitative polymerase chain reaction. In addition, levels of cytokines including interferon gamma (*IFN-γ*), interleukin (*IL*)-2, *IL*-6, *IL*-4, and *IL*-10 were quantified using enzyme-linked immunosorbent assay. The right ovaries were examined histologically and immunohistochemically using hematoxylin and eosin staining and *NF-κB* immunostaining.

**Results:** Malathion exposure significantly increased the *Bax/Bcl-2* ratio, upregulated *Bax* and *NF-κB* expression, and elevated levels of *IFN-γ*, *IL*-2, and *IL*-6. It also enhanced miRNA-146a-5p expression, while reducing miRNA-129-3p and miRNA-96-5p levels, as well as *IL*-4 and *IL*-10. Histological analysis revealed that malathion induced ovarian structural abnormalities and disrupted follicular architecture, accompanied by increased *NF-κB* immunoreactivity. Conversely, hesperidin treatment demonstrated a protective role by

alleviating or reversing these molecular and structural alterations.

**Conclusion:** In conclusion, hesperidin ameliorates malathion-induced ovarian toxicity by modulating cytokine production, apoptotic signaling, inflammatory responses, and miRNA expression.

**Keywords:** Ovary, Malathion, Hesperidin, Apoptosis, Inflammation, miRNAs.

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## P-114

### Possible regulatory role of circulating miRNA-708 in endometriosis

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**Background:** Endometriosis is a chronic, estrogen-dependent condition that affects women of reproductive age. It is characterized by the presence of endometrial-like tissue outside the uterine cavity. A key challenge in managing endometriosis is the absence of a reliable noninvasive diagnostic test, often resulting in delayed diagnosis and treatment. Implementing noninvasive diagnostic techniques could facilitate early identification and intervention, potentially enhancing the quality of life for affected women and mitigating related healthcare costs. Despite the high prevalence of endometriosis, the underlying factors and mechanisms that drive the formation and persistence of ectopic lesions remain poorly understood. Therefore, alongside conventional treatment strategies, it is crucial to explore the genetic determinants of the disease, particularly the alterations in microRNA (miRNA) expression, which may elucidate its etiology and inform therapeutic approaches.

**Objective:** Comparing the miRNAs expression among women with endometriosis relative to the control group.

**Materials and Methods:** This case-control study involved collecting plasma samples from 20 women with endometriosis who underwent laparoscopic surgery at Research and Clinical Center for Infertility, Yazd, Iran in 2022, alongside 20 women without endometriosis. Total RNA was extracted from plasma samples using trizol, followed by complementary DNA (cDNA) synthesis. The synthesized cDNA served as a template for real-time polymerase chain reaction analysis. The 2<sup>-ΔΔCt</sup> method was employed to assess the expression level changes of miR-708.

**Results:** Comparative analysis of miR-708 expression levels in plasma revealed a significant decrease in both miRNAs among women with endometriosis relative to the control group. The ability of miR-708 to accurately identify women with endometriosis was measured using ROC curves and the area under the curve. The area under the curve value for miR-708 was 0.68 ( $p = 0.048$ ) for plasma samples when compared to the plasma of the control group.

**Conclusion:** The significant differences in the expression of these candidate miRs between the plasma of endometriosis participants and healthy individuals suggest their potential involvement in the etiology of endometriosis. Consequently, miR-708 may serve as prospective diagnostic biomarkers and could play a role in prognosis and treatment management.

**Keywords:** Endometriosis, Circulating miRNA, miR-708, Plasma.

### P-115

#### Investigation of the *SPATA6* gene expression in men with terato-asthenozoospermia

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**Background:** Infertility has emerged as a significant issue in contemporary society, with its prevalence on the rise globally. A proportion of men suffer from a condition referred to as idiopathic male infertility, which is marked by an ambiguous cause of the disorder. The spermatogenesis-associated protein (*SPATA*) gene family is responsible for encoding proteins that are crucial for the processes of spermatogenesis and fertilization. Within this family, the *SPATA6* gene is notably conserved throughout evolution and exhibits expression that is restricted to testicular tissue.

**Objective:** In the present study, aimed to examine the expression level of the *SPATA6* in individuals with terato-asthenozoospermia (TAZ) and Normozoosperma (NZ) as a control group.

**Materials and Methods:** This research, conducted in collaboration with the Shahrekord Infertility Treatment Center and the Clinical Research Development Unit of Hajar hospital at Shahrekord University of Medical Sciences, Shahrekord, Iran from July 2023 until Jan 2024, involves a study sample comprising individuals diagnosed with TAZ ( $n = 30$ ) alongside a control group of NZ with normal seminal fluid parameters ( $n = 30$ ). Seminal fluid samples are collected from the participants under standardized conditions. The expression level of the gene was evaluated by real-time polymerase chain reaction.

**Results:** The findings of this study indicated that the expression level of *SPATA6* was significantly ( $p <$

0.0001) lower in the TAZ group than in the control group. There were also significant ( $p < 0.0001$ ) positive associations between the expressions of *SPATA6* with sperm motility and morphology.

**Conclusion:** Overall, the results of this study suggested the role of the *SPATA6* gene in sperm motility and morphology.

**Keywords:** Male infertility, *SPATA6*, Asthenozoospermia, Gene expression.

### P-116

#### Integration of artificial intelligence and genomic editing: A novel revolution in infertility diagnosis and treatment

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**Background:** Artificial intelligence and genomic editing technologies, particularly clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9), are aiding in reducing infertility, a genetic disorder affecting millions globally. Researchers are developing new treatment strategies and addressing societal responsibilities and ethical issues.

**Materials and Methods:** This 2024 systematic review examined English-language peer-reviewed articles from 2023-2024 on infertility, genomic editing, personalized treatment, AI, CRISPR-Cas9, and ethical considerations. Searches in Web of Science, PubMed, Medline, Embase, and Google Scholar identified 67 articles. After screening titles (40 articles), abstracts (15 articles), and full texts (7 excluded), 8 articles were selected for final analysis.

**Results:** The use of (AI) in infertility diagnostics allows for accurate diagnosis and prognostication in cases of infertility due to its remarkable ability to evaluate big and complicated datasets. For example, machine learning algorithms determine the best intervention tactics and forecast the success rates of assisted reproductive technologies, such as in vitro fertilization. Additionally, AI-powered solutions produce thorough fertility profiles, pinpoint risk factors, and offer practical suggestions for changing one's lifestyle, enabling prompt and efficient interventions. CRISPR and genomic editing: CRISPR-Cas9 provides unmatched accuracy in modifying infertility-related genomic sequences. This method has demonstrated promise in correcting mutations associated with ovarian insufficiency and azoospermia. By anticipating off-target effects, improving editing techniques, and customizing genomic interventions, the incorporation of AI increases the effectiveness of CRISPR. Previously untreatable cases of infertility can now be resolved because of these developments. Moral points to remember: there are serious ethical issues with the use of AI and CRISPR in infertility treatment. To guarantee fair access and lessen inequities in healthcare, concerns

including patient autonomy, informed consent, and data security must be addressed. Creating strong ethical frameworks is essential to strike a balance between societal responsibility and innovation. Therapeutic synergies: AI and CRISPR together have demonstrated revolutionary potential in the treatment of infertility. CRISPR-based therapies are made more accurate and safer by AI-driven insights. The care of complicated infertility cases is being revolutionized by this synergistic approach, which makes it easier to generate customized genetic therapies.

**Conclusion:** AI and CRISPR are revolutionizing infertility treatment by improving diagnosis and treatment effectiveness. AI enhances CRISPR editing capabilities by analyzing large datasets. However, adoption faces challenges like high costs, accessibility, and ethics concerns. Future studies aim to enhance these technologies and explore their long-term effects.

**Keywords:** Artificial intelligence, CRISPR-Cas9, Infertility, Genomic editing, Personalized treatment, Ethical considerations.

### P-117

#### Differential expression in female with endometriosis through single-cell RNA sequencing

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**Background:** Endometriosis is a chronic and often debilitating gynecological condition characterized by the presence of endometrial-like tissue outside the uterine cavity. Affecting an estimated 10-15% of women of reproductive age, this disorder presents significant clinical challenges, including chronic pelvic pain and infertility, thereby adversely impacting quality of life. There is a challenge among scientists regarding whether endometriosis affects oocyte quality, implantation, and pregnancy outcomes. Understanding these relationships is crucial for developing effective treatment strategies for individuals affected by endometriosis. The target of this study is to investigate the impact of endometriosis on oocyte quality by analyzing the differential gene expression in oocytes from women with endometriosis compared to healthy individuals.

**Objective:** The study aims to identify key genes, such as *HOPX*, *RHOB*, *RRAD*, and *GADD45G*, that are differentially expressed and may play critical roles in oocyte quality and the reproductive pathophysiology associated with endometriosis. Ultimately, this research seeks to elucidate the mechanisms by which these genes influence oocyte biology and to explore their potential as biomarkers or therapeutic targets for improving

fertility treatment outcomes in women with endometriosis.

**Materials and Methods:** In this case-control study raw data excluded from GEO datasets of NCBI. 4 samples are the RNA-sequencing of oocyte from endometriosis individuals and four sample is the RNA-seq of oocyte from healthy individuals. The quality of data assessed by FASTQC in GALEXY. Then data mapped to the genome by HISAT2. After assessing the quality of annealing gene expression assessed by DEseq2 twenty top genes with  $p < 0.05$  and adjusted  $p > 1$  was selected and differential gene expression of samples was compared by heatmap. Our scRNA-seq revealed an effect of endometriosis on global transcriptome behavior in oocyte from endometriosis ovaries.

**Results:** In this study, MA plot, histogram of p-value, dispersion estimates of normalized counts, PC variance and sample to sample distance thoroughly examined by DEseq2 and all exhibited an acceptable level of quality. The highest number of differentially expressed genes was found when oocyte from women with OE were compared to oocyte from healthy donors. In this study the gene *HOPX*, *RHOB*, *RRAD* and *GADD45G* is so expressed in oocyte from endometriosis individual compared to oocyte from healthy donors.

**Conclusion:** The altered expression of genes like *HOPX*, *RRAD*, *RHOB*, and *GADD25G* in oocytes from individuals with endometriosis points to their importance in oocyte quality and endometriosis-related reproductive issues. These genes affect key processes like stress response and metabolism, and their disruption can impair oocyte function, contributing to infertility. Further research is needed to fully understand how these genes impact oocyte biology and whether they could serve as targets for future treatments.

**Keywords:** Endometriosis, Oocyte, Single cell RNA-sequencing, Bioinformatic, Infertility.

### P-118

#### Effect of platelet-rich plasma therapy on the expression of TRAF-1 and COX-2 in the endometrium

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**Background:** Platelet-rich plasma (PRP) is plasma from whole blood that has a high concentration of platelets, which includes various growth factors that can promote tissue healing through stimulating cell growth, forming new blood vessels, and reducing inflammation. The TNF receptor-associated factor (TRAF) family consists of cytoplasmic proteins that can negatively regulate apoptosis and also induce genes that enhance cell survival. TRAF proteins might function to regulate the ability of receptors to activate different signaling pathways, resulting in the phosphorylation and

activation of protein kinases, which subsequently leads to the activation of rel and AP-1 family transcription factors. TRAFs are recognized as downstream signal transmitters of the tumour necrosis factor (TNF) receptor family. Endometritis in Jennies correlates with an oxidative process, changes in serum biochemical parameters, Doppler indices, endoscopic appearance, elevated *NF-κB* expression, and increased levels of TRAF-1. Uterine inflammation is a natural response that takes place following artificial insemination or natural mating to eliminate surplus semen and microorganisms from the uterine cavity. Moreover, the acute inflammation process starts following the detection of bacteria or semen by the toll-like receptors located in the endometrial cells. Upon toll-like receptors activation, nuclear factor-kappa B is produced, leading to the activation of proinflammatory cytokines, chemokines, and cyclooxygenase-2 (COX-2). These molecules control the inflammatory signals to the immune cells.

**Objective:** This study aimed to investigate the effect of PRP on several specific factors levels in the endometrial tissue including *COX-2* and *TRAF-1*.

**Materials and Methods:** We investigated several studies from January 2015-2025. These were explored in Web of science, Pubmed and Scopus. Also, used keywords were including: Platelet-rich plasma, Endometrial, *COX-2*, and *TRAF-1*. An inclusion criterion was English language and exclusion cases were non-english language and retracted studies. A total of 58 primary articles were initially retrieved from journals indexed in Web of Science, Scopus, and PubMed. Following the removal of duplicate entries, 31 distinct articles were retained for further evaluation. After meticulous scrutiny of titles and abstracts, 11 articles were ultimately selected, while 4 records were excluded due to various reasons, such as non-reporting of the relevant outcome. Finally, 7 studies were retained for the final analysis.

**Results:** PRP effectively decreases the inflammatory reaction in mares with persistent mating-induced endometritis regardless of the timing of treatment, thereby enhancing the likelihood of a successful pregnancy. Both PRP therapies led to a reduction in polymorphonuclear neutrophils in the cytology. The number of horses with endometritis decreased in the treatment groups, and the control group showed a higher level of positive *COX-2* labeling compared to the 2 treatment groups. Following intrauterine PRP infusion, there was a gradual significant decrease in *TRAF-1* gene expression. Similarly, the *MUC-1* gene expression also gradually decreased after intrauterine PRP infusion. Both genes remained within normal levels.

**Conclusion:** The effect of PRP is decreasing the inflammatory reaction in addition to a gradual significant decrease in *TRAF-1* and *MUC-1* genes.

**Keywords:** Platelet-rich plasma, Endometrial, *COX-2*, *TRAF-1*.

#### P-119

**Frozen cell fate: Investigating the epigenetic changes in ovarian and testicular tissues post-cryopreservation**

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Cryopreservation is one of the effective approaches for the preservation of fertility in men and women, especially in those whose fertility is threatened by disease. However, challenges such as the risk of epigenetic instability caused by cryopreservation remain, which is still controversial. The study aimed to investigate the epigenetic effects of cryopreservation on ovarian and testicular tissues. The present study analyzed the literature on cryopreservation of oocytes, embryos, and testicular tissue. Comprehensive searches were conducted in databases such as PubMed, Scopus, and Web of Science. In this regard, studies have revealed that freezing can alter gene expression and protein abundance and affect epigenetically relevant factors. For example, preclinical studies have revealed that vitrification and warming of juvenile mouse ovaries reduces mRNA and protein levels of DNMT1. Also, it can alter the expression of epigenetically important genes like *DNMT3B* and *HDAC1*. In addition, cryopreservation affects imprinted genes such as *Igf2r*, *H19*, and *PLAGL1* in murine ovarian tissue. Regarding testicular tissues post-cryopreservation, it has been revealed that cryopreservation can lead to epigenetic instability, especially affecting imprinted genes like *H19*, *PEG3*, and *KCNQ1OT*. Additionally, regarding post-translational histone modifications, some studies report no significant changes after freezing. It is while some others report a reduction in specific histone marks. Nevertheless, the complexity of epigenomic control highlights the need for caution in interpreting findings, especially regarding the potential for de novo epimutations associated with cryopreservation protocols involving dimethyl sulfoxide and enzyme treatments. All in all, it can be stated that investigations of epigenomic changes after ovarian tissue cryopreservation have shown various results. Some studies report minor effects, while others highlight potential imprinting defects associated with using cryopreservation techniques. Although some approaches for the treatment of male fertility via testicular tissue cryopreservation are promising, they remain experimental and further research is needed to assess long-term epigenetic effects and potential epimutations in both sexes.

**Keywords:** Cell fate, Cryopreservation, Genomic imprinting, Epigenetics.

#### P-120

**Recent findings on a role of novel circular RNAs in infertility**

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Reproduction involves numerous biological processes including meiosis, mitosis, hormone synthesis, neuroendocrine regulation, and development of reproductive organs. Infertility, defined as the inability to achieve pregnancy after 12 months of unprotected intercourse, affects approximately one in seven couples worldwide and poses a major challenge to global reproductive health. This narrative review aims to investigate recent findings regarding the role of circular RNAs (circRNAs) in the pathogenesis of infertility in both males and females. We performed a narrative review of literature published from 2015-2024 using PubMed, Scopus, and Web of Science databases. Keywords including "circular RNA", "infertility", "reproduction", and "assisted reproductive technology" were used. Articles were screened based on relevance, originality, and scientific quality. Both experimental and clinical studies investigating the functional roles of circRNAs in reproductive biology and infertility were included. circRNAs, a class of endogenous non-coding RNAs with covalently closed circular structures, were initially regarded as non-functional byproducts of transcription. However, recent studies have revealed their significant roles in gene regulation through various mechanisms. CircRNAs have been shown to influence gametogenesis, embryo development, ovarian aging, polycystic ovary syndrome, gynecologic malignancies, and pregnancy-related complications. Their potential as biomarkers and therapeutic targets in reproductive medicine is increasingly recognized. Additionally, the application of gene-editing technologies such as CRISPR-associated protein 9 has contributed to the identification of novel functions for circRNAs. In assisted reproductive technology, profiling circRNAs may offer insights into gamete and embryo quality, possibly improving assisted reproductive technology outcomes. CircRNAs have emerged as critical regulators of reproductive function and may contribute to the pathophysiology of infertility. Further investigations into their molecular mechanisms could lead to novel diagnostic and therapeutic strategies in reproductive medicine.

**Keywords:** Infertility, Circular RNAs, Reproduction, Assisted reproductive technology, Non-coding RNA.

### P-121

#### **A review of the impact of hormones and genetic mutations on fertility health: Innovative approaches to infertility treatment**

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Infertility is a complex condition that can be caused by a range of hormonal, genetic, and structural factors. This

article examines the critical roles that hormones and genetic mutations play in both male and female fertility. Hormones such as anti-Müllerian hormone, testosterone, estrogen, prolactin, progesterone, thyroid-stimulating hormone, and gonadotropins are pivotal in regulating reproductive functions, including ovulation, menstrual cycles, and pregnancy. Variations in the levels of these hormones can lead to a variety of fertility disorders. For example, high levels of prolactin or altered levels of thyroid hormones can disrupt ovulation, while imbalances in estrogen and progesterone can interfere with embryo implantation and pregnancy maintenance. Genetic factors also play a significant role in fertility. Mutations in genes such as follicle-stimulating hormone receptor and peroxisome proliferator-activated receptor Gamma have been associated with reproductive dysfunctions, including premature ovarian failure, polycystic ovary syndrome, and male infertility. Polymorphisms in these genes can affect hormone receptor sensitivity and gene expression, leading to impaired ovarian function or sperm production. Recent advances in infertility treatments have focused on both genetic and hormonal regulation, offering new hope for participants with fertility challenges. For example, hydrogels derived from the extracellular matrix have shown promise in reconstructing the endometrium and improving embryo implantation rates. These hydrogels mimic the natural uterine environment and help support the rebuilding of the uterine lining, offering a potential solution for women with thin endometria or implantation failure. Furthermore, new developments in in vitro maturation offer a promising, less invasive alternative to traditional in vitro fertilization by improving oocyte quality and embryo development with minimal use of hormonal treatments. Additionally, the use of growth factors like cumulin and signaling molecules such as cAMP has been shown to enhance oocyte maturation, improving both oocyte quality and embryo viability without the need for extensive hormone stimulation. This approach has the added benefit of reducing the risk of ovarian hyperstimulation syndrome, a common complication in traditional in vitro fertilization treatments. These breakthroughs represent significant progress in fertility treatment, offering more personalized, less invasive, and cost-effective options for participants. With further research, these innovative therapies could lead to more successful fertility treatments, especially for women with endometrial disorders, hormonal imbalances, or those at risk of ovarian hyperstimulation syndrome. The combination of hormonal regulation, genetic screening, and advanced biomaterials offers a multi-faceted approach to addressing infertility and may pave the way for more efficient and tailored treatments in the future.

**Keywords:** Anti-Müllerian hormone, FSHR, In vitro maturation, Ovarian hyperstimulation syndrome, IVF.

### P-122

#### **Frequency of *ESR1* Gene polymorphisms *rs2234693* and *rs9340799* in women with recurrent pregnancy loss: A case-control study**

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**Background:** Recurrent pregnancy loss (RPL) is a multifactorial condition that may involve genetic predispositions, including polymorphisms in estrogen receptor genes (ESR).

**Objective:** The present study aimed to investigate the frequency of two common *ESR1* gene polymorphisms, *rs2234693* and *rs9340799*, in women with a history of RPL compared to healthy controls.

**Materials and Methods:** This case-control study was conducted on 100 women with a history of RPL and 100 healthy women without such history. Peripheral blood samples were collected, genomic DNA was extracted, and genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism method. Hardy-Weinberg equilibrium was assessed in the control group for both polymorphisms.

**Results:** The genotype distribution of both polymorphisms in the control group was in accordance with Hardy-Weinberg equilibrium. A significant association was observed between the *rs2234693* polymorphism and RPL, with both the CC genotype and C allele being more frequent in the RPL group. However, no significant association was found between *rs9340799* and RPL, although the GG genotype was slightly more prevalent in the RPL group, it did not reach statistical significance.

**Conclusion:** The findings suggest a possible role of the *rs2234693* polymorphism of the *ESR1* gene in the etiology of RPL. No meaningful association was found for *rs9340799*. It is important to consider ethnic and population-specific genetic backgrounds when studying polymorphism-related disease susceptibility. This study was conducted in Yazd, Iran, and contributes to the growing body of population-based genetic research on RPL.

**Keywords:** Recurrent pregnancy loss, *ESR1* gene, *rs2234693*, *rs9340799*, Polymorphism, PCR-RFLP.

## P-123

### Expression of pre-meiotic and post-meiotic markers during in vitro spermatogenesis on testicular extracellular matrix-gelatin scaffold

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**Background:** Male infertility treatments continue to pose significant challenges, with limited options available. Recently, tissue engineering has provided new opportunities to direct the behavior of spermatogonial stem cells (SSCs) during in vitro spermatogenesis in 3-dimensional microenvironments.

**Objective:** This study aims to evaluate the viability and differentiation of SSCs on a porous scaffold derived from testicular extracellular matrix (TECM).

**Materials and Methods:** In this experimental study, testicular tissue from 5 rams (1 yr) was processed through decellularization to obtain TECM, which was then combined with gelatin to fabricate a hybrid scaffold using the gas foaming technique. Testicular cells were isolated from the testes of neonatal mice, and the presence of SSCs was confirmed using flow cytometry for the specific marker *Plzf*. Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were cultured on scaffold and induced to assess the differentiation process for 4 wk. The presence of pre-meiotic and post-meiotic cells in the experimental (TECM-gelatin) and control (gelatin) groups was evaluated through real-time quantitative reverse transcription polymerase chain reaction and flow cytometry techniques.

**Results:** According to our results, no significant differences in cell survival were observed between the 2 groups during the 7- and 14-day post-incubation periods. After 4 wk of culture, the expression of pre-meiotic genes (*Plzf*, *Id4*) and post-meiotic genes (*Sycp3*, *Prm1*) in the TECM-gelatin scaffold was significantly higher than that in the gelatin-only scaffold, as determined by real-time quantitative reverse transcription polymerase chain reaction ( $p < 0.001$ ). Additionally, flow cytometry showed more *sycp3*-positive and acrosin-positive (post-meiotic) cells in the TECM-gelatin scaffold compared to the gelatin scaffold ( $p < 0.001$ ).

**Conclusion:** Our findings indicate that the TECM-gelatin scaffold effectively supports the proliferation and differentiation of SSCs, suggesting its potential as a valuable approach for testicular tissue engineering.

**Keywords:** Testis, Extracellular matrix, Spermatogonial stem cells, Differentiation, Gas foaming method.

## P-124

### Evaluation of liposome containing heder helix extract and miRNA-124a cytotoxicity in breast cancer cell line (MCF-7)

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**Background:** Breast cancer is one of the most prevalent malignancies among women worldwide and remains a major cause of cancer-related mortality. miRNA-124 is recognized as a tumor suppressor because it regulates key oncogenic pathways. Herbal derivation like Heder helix extract (HHE) is another alternative cancer treatment with low side effects; however, using it because of its low solubility and high effective dose faces limitations. Liposomal nanocarriers have emerged as promising vehicles for miRNA-based therapies and drug delivery carriers.

**Objective:** This experimental study aimed to evaluate the efficacy of a liposomal formulation for miRNA-124 containing HHE delivery in breast cancer cells (MCF-7 cells), comparing its impact on cell viability with that of free miRNA-124a and the free form of the extract.

**Materials and Methods:** Various liposomal formulations were synthesized using the thin-film hydration method, followed by extrusion to obtain uniform vesicles. In this experimental study, a specific molar ratio of lipid components (5%) was used to optimize the liposome composition for enhanced stability and cellular uptake. The extract was loaded into nanocarriers by using the hydration method. miRNA-124a was loaded into the liposomes using an optimized N/P ratio (1.50) by incubation at 37°C. Characterization of liposomal formulation, dynamic light scattering analysis, scanning electron microscopy analysis, atomic force microscopy analysis, drug release rate (48 hr), and encapsulation efficiency. Breast cancer cell culture and treatment, human breast cancer cell lines MCF-7 were cultured for 48 hr. After 48 hr of treatment, cell viability was assessed using the MTT assay ( $p < 0.005$ ).

**Results:** The drug load (63%) and miRNA binding of synthesized nanoparticles were appropriate. The results of microscopy techniques also demonstrated that the nanoparticles had homogenized smooth surfaces without any aggregation. This result was aligned with the result of dynamic light scattering, which showed that the surface charge of the nano-system was tiny positive ( $5.2 \pm 1.4$  mV), and its size was also suitable ( $94.3 \pm 4.1$  nm). MTT results demonstrated that the cytotoxicity of the system was significantly ( $52.3\% \pm 2.3$ ) higher than

that of the free form of the extract and miRNA, which proved its efficiency for cancer treatment.

**Conclusion:** This study showed that the synthesized liposome can remarkably increase the cytotoxicity of the HHE which indicates its efficiency, and also showed that using miRNA-124a can increase the apoptosis of cancerous cells by affecting their molecular pathways. So, using this nanosystem for breast cancer treatment can open new windows toward a certain treatment with low side effects.

**Keywords:** Breast cancer, Liposome, miRNAs, Heder helix, MTT, MCF-7.

## P-125

### Evaluation of the expression levels of FZD3 and miR-378 in women with polycystic ovarian syndrome

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**Background:** Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women of reproductive age. The cause of PCOS is unknown. Studies have shown that some genes and miRNAs, such as *FZD3* and miR-378, play a role in infertility and cancer. Recent studies suggest that Wnt/FZD signaling plays a crucial role in regulating granulosa cell function and oocyte-cumulus communication, both vital for oocyte maturation. Dysregulation of *FZD3* expression or its downstream pathways may lead to impaired maturation, affecting fertility outcomes.

**Objective:** Considering the importance of *FZD3* and miR-378 in ovulation, the present study aimed to determine the expression levels of *FZD3* and *miR-378* genes in cumulus cells of germinal vesicle and metaphase II oocytes in women with PCOS.

**Materials and Methods:** In this case-control study, cumulus cells were collected from 25 randomly selected women diagnosed with PCOS at the Yazd Research and Clinical Center for Infertility, Yazd, Iran between March 2023 and May 2024. The diagnosis of PCOS was based on the criteria outlined in the Rotterdam guidelines. The expression levels of *FZD3* and miR-378 were determined using real-time polymerase chain reaction.

**Results:** This study demonstrated a higher expression of *FZD3* and miR-378 in cumulus cells at the germinal vesicle stage compared to metaphase II oocytes ( $p < 0.0001$ ).

**Conclusion:** Excessively high levels of *FZD3* and miR-378 in the cumulus cells of immature oocytes can hinder their maturation. *FZD3*, a component of the Wnt signaling pathway, is overexpressed in immature oocytes and may negatively impact their maturation process. Additionally, miR-378 inhibits oocyte

development by targeting and suppressing essential genes. These findings are significant for women with PCOS.

**Keywords:** PCOS, FZD3, miR-378, RTqPCR.

#### P-126

### Investigating the effect of hydroalcoholic extract of *Taverniera Spartea* on the AKT/mTOR/ $\beta$ -Catenin signaling pathway in triple negative breast cancer cells

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**Background:** In general, it is difficult to increase and evaluate new markers towards breast most cancers because of its extreme organic heterogeneity. Triple tumors are proof against therapeutic methods and may recur and broaden metastatic abilities. Consequently, there is an urgent want to discover and develop new capability therapeutic agents towards them.

**Objective:** Considering the known anticancer effects of silver spruce, here we aimed to investigate the effect of this plant on the AKT (protein kinase B) /mTOR (mammalian target of rapamycin) / $\beta$ -catenin signaling pathway in triple negative breast cancer cells in order to study the mechanism of action of this plant.

**Materials and Methods:** In this experimental study, hydroalcoholic extract of the plant was prepared and breast cancer cell line was treated with different concentrations of the extract. Cell survival was evaluated by MTT (3-[4, 5-dimethylthiazolyl-2]-2, 5-diphenyltetrazolium bromide) assay. Apoptosis was examined by diphenylamine method. Finally, real-time polymerase chain reaction technique was used to measure the expression of hypoxanthine guanine phosphoribosyl transferase, osteopontin, protein kinase B, mammalian target of rapamycin,  $\beta$ -catenin genes.

**Results:** After 24, 48, 72 and 96 hr of treatment, cell viability decreased in a concentration- and time-dependent manner. The IC<sub>50</sub> values for 24, 48, 72 and 96 hr were  $1266.13 \pm 8.47$ ,  $865.86 \pm 11.52$ ,  $431.4 \pm 27$  and  $98.79 \pm 5.88$   $\mu$ g/ml, respectively. Also, the toxicity of the extract increased in a concentration- and time-dependent manner. The hydroalcoholic extract after 24 hr at the IC<sub>50</sub> concentration caused a significant increase in apoptosis ( $p < 0.001$ ). Also, a significant decrease in the expression of hypoxanthine guanine phosphoribosyltransferase, osteopontin, protein kinase B, mTOR,  $\beta$ -catenin genes was observed ( $p < 0.001$ ).

**Conclusion:** Hydroalcoholic extract of silver spruce tree reduces viability and induces apoptosis in triple-negative breast cancer cells by disrupting different stages of Wnt (wingless-type MMTV integration site / $\beta$ -catenin) signaling.

**Keywords:** *Taverniera spartea*, Breast cancer, Apoptosis.

#### P-127

### Identifying a new mutation (c.550delG) in exon 5 of the *AURKC* gene and its association with macrozoospermia

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**Background:** Macrozoospermia is a rare disorder characterized by large-headed, multi-flagellated spermatozoa, resulting in male infertility. The *AURKC* gene, located on chromosome 19, encodes a serine/threonine protein kinase critical for meiotic regulation and chromosome segregation. This gene's mutation may disrupt the protein's function and lead to abnormal sperm production.

**Objective:** This study investigated genetic variants in exon 5 of the *AURKC* gene among men with macrozoospermia referred to the Royan Research Institute, Tehran, Iran.

**Material and Methods:** This case-control study was conducted at the infertility department of Royan Research Institute, Tehran, Iran between September 2022 and August 2023. The case group included 10 infertile men diagnosed with macrozoospermia (> 15% of sperm with large heads), while the control group consisted of 10 fertile men with normal semen analyses and a history of successful fertility, selected from the institute's clients. The inclusion criteria for both groups were based on World Health Organization standards. To assess sperm morphology, semen samples were examined under a light microscope after Papanicolaou staining. Genomic DNA was extracted from peripheral blood samples. Polymerase chain reaction and then Sanger sequencing were performed to detect the presence of the mutation. The results were analyzed using Finch TV and Nucleotide Blast. Bioinformatics tools, mutation taster and sorting intolerant from tolerant, were also used to assess the biological effects of the identified mutations.

**Results:** A novel mutation (c.550delG; p.D184I fs10) in exon 5 of the *AURKC* gene was identified in 1 out of 10 patients with macrozoospermia, whereas none of the 10 controls carried this mutation. Bioinformatics analysis showed that this mutation, by generating a premature stop codon, activates the nonsense-mediated decay pathway, which increases the likelihood of mRNA degradation and protein instability.

**Conclusion:** In this study, a novel mutation (c.550delG; p.D184I fs10) was identified in exon 5 of the *AURKC* gene in one patient with macrozoospermia. Bioinformatic analyses using sorting intolerant from tolerant and mutation taster tools indicate that this mutation may have significant effects on the expression and function of the protein and has been classified as a deleterious mutation.

**Keywords:** Male infertility, Aurora kinases, Mutation.

## P-128

### Identification of novel cytoskeleton protein involved in sperm and sertoli cells of non-obstructive azoospermia based on microarray and bioinformatics analysis

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**Background:** Non-obstructive azoospermia (NOA) is a severe form of male infertility characterized by a complete absence of sperm in the ejaculate due to impaired spermatogenesis.

**Objective:** To strengthen the reliability of our observations, we cross-referenced our data with publicly available single-cell genomics datasets.

**Materials and Methods:** The cytoskeleton, scaffold, and actin-binding genes were analyzed by microarray and bioinformatics (771 spermatogenic cell genes and 774 Sertoli cell genes). To validate cross-sectional study findings, we cross-referenced our results with data from a single-cell genomics database (GSE216907 and GSE235324).

**Results:** In the microarray analyses of 3 human cases with different NOA spermatogenic cells and 3 case of normal cells, the expression of *TBL3*, *MAGEA8*, *KRTAP3-2*, *KRT35*, *VCAN*, *MYO19*, *FBLN2*, *SH3RF1*, *ACTR3B*, *STRC*, *THBS4*, and *CTNND2* were upregulated, while expression of *NTN1*, *ITGA1*, *GJB1*, *CAPZA1*, *SEPTIN8*, and *GOLGA6L6* were downregulated. There was an increase in *KIRREL3*, *TLL9*, *GJA1*, *ASB1*, and *RGPD5* expression in the Sertoli cells of three human cases with NOA, whereas expression of *DES*, *EPB41L2*, *KCTD13*, *KLHL8*, *TRIOBP*, *ECM2*, *DVL3*, *ARMC10*, *KIF23*, *SNX4*, *KLHL12*, *PACSIN2*, *ANLN*, *WDR90*, *STMN1*, *CYTA*, and *LTBP3* were downregulated. A combined analysis of Gene Ontology (GO) and STRING, were 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, and positive regulation of cell-substrate adhesion were significantly associated with differentially expressed genes (DEGs) in spermatogenic cells. Molecular function (MF) of DEGs that were up/down regulated, it was found that tubulin bindings, gap junction channels, and tripeptide transmembrane transport were more significant in our analysis. An analysis of GO enrichment findings of Sertoli cells showed BP and MF to be common DEGs. Cell-cell junction assembly, cell-matrix adhesion, and regulation of SNARE complex assembly were significantly correlated with common DEGs for BP. In the study of MF, U3 snoRNA binding, and cadherin binding were significantly associated with common DEGs.

**Conclusion:** Our analysis, leveraging single-cell data, substantiated our findings, demonstrating significant alterations in gene expression patterns.

**Keywords:** Non-obstructive azoospermia, Microarray, Spermatogenic cells, Sertoli cell, Cytoskeleton protein.

## P-129

### In silico analysis and experimental validation of imprinting gene expression (*RASGRF1* and *NTM*) in endometrium of recurrent implantation failure

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**Background:** Recurrent implantation failure (RIF) and recurrent spontaneous abortion (RSA) represent intricate issues within the realm of assisted reproductive technology, and their underlying causes are often linked to compromised endometrial receptivity.

**Objective:** Eight microarray datasets related to RIF and RSA were retrieved from the Gene Expression Omnibus database and were subsequently integrated.

**Materials and Methods:** To finalize the cross-sectional study, hub genes, miRNA, and long non-coding RNAs (lncRNA) with imprinting significance were pinpointed through the application of CytoHubba. In the endometrial tissue of individuals suffering from 5 RIF and 5 RSA (collected at Royan Institute, Tehran, Iran from 2023-2024), a noteworthy differential expression was observed in 33 genes, 49 microRNAs, and 137 lncRNA that are associated with imprinted gene regulation.

**Results:** Through a meticulous functional enrichment analysis, it became evident that the altered gene expression profile in these 2 groups primarily pertained to significant modifications in biological processes. Specifically, these changes were predominantly linked to the G-protein coupled receptor signaling pathway, the regulation of interleukin-1 beta production, and the binding of phosphatidylinositol bisphosphate within the endometrial tissue. Furthermore, a subset of key differentially expressed genes and single-cell *RASGRF1*, *NTM*, and *KBTBD3* were identified as hub genes implicated in the pathogenesis of RIF, while *KBTBD3*, *PTPN14*, and *DHCR7* were singled out as hub genes specifically associated with RSA.

**Conclusion:** The real-time polymerase chain reaction validation shows that *RASGRF1*, *NTM*, and *KBTBD3* are significantly changed.

**Keywords:** In silico, Imprinting gene, Non-coding RNA, Recurrent implantation failure.

**The original full text of this abstract has been published:**

Hashemi Karoii D, Azizi H, Darvari M, Qorbanee A, Hawezy DJ. Identification of novel cytoskeleton protein involved in spermatogenic cells and sertoli cells of non-obstructive azoospermia based on microarray and bioinformatics analysis. *BMC Med Genom* 2025; 18: 19.

### P-130

#### **Integrative transcriptomic analysis of endometrial tissue: Unveiling novel biomarkers for endometriosis diagnosis**

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**Background:** Endometriosis affects an estimated 190 million women and individuals assigned female at birth globally. This chronic, inflammatory gynecological condition is characterized by the presence of tissue resembling the endometrium outside the uterus. Many individuals with endometriosis experience severe and debilitating pain. Additionally, the condition increases the risk of infertility, fatigue, widespread pain, and other associated health issues. Endometriosis presents with diverse symptoms and impacts various stages of life. Delayed diagnosis following the onset of symptoms is a frequent challenge, and symptoms often persist or recur even after treatment.

**Objective:** This study utilizes transcriptomic analysis to explore differences in gene expression between individuals with endometriosis and healthy controls. Overall, we demonstrated that estrogen/estrogen receptor- $\beta$  and prostaglandin integrate at as-like, estrogen-regulated, growth inhibitor, leading to increased endometriotic cell proliferation and represents a novel candidate for therapeutic intervention.

**Materials and Methods:** This study also performed an analysis of high-throughput microarray data and differential expressed genes (DEGs) based on endometrial tissue samples. Gene expression datasets were collected from Gene Expression Omnibus (GEO) database statistical analyses were performed to assess gene expression in endometriotic tissues and various plots were generated for visualization using R programming.

**Results:** By analyzing the graphs, we found that some genes have significant gene expression changes, while others do not have significant gene expression changes. DGE: a whole lot of genes that are statistically meaningful (Padj < 0.025) and this consist of the upregulated genes and downregulated genes seen in MA and Volcano plots. Likely participating in the biological mechanisms of endometriosis, these DEGs may prove useful as novel biomarkers or therapeutic targets. Distinctive group clustering: dimensionality reduction methods (e.g., UMAP) show clear separation between case and control groups, indicating that the transcriptomic profiles can successfully separate these populations. This supports the identification of DEGs that mirror disease-specific features. Expression trends:

The expression density plot and box plot were used to analyze the overall normalization of gene expression across the dataset with little technical variation. Yet there are significant expression differences in some genes in cases versus controls, underscoring their biological relevance. Pathway and functional insight: genes demonstrating significant expression changes (QQ and Volcano plots) are likely connected with key pathways that are functional in endometriosis. In the disease processes, the pathway enrichment analyses (GO or KEGG) are equally compelling but must be carried out to reveal their functional significances. The DEGs identified in this study have potential to be both validated as biomarkers and therapeutic targets for endometriosis. To further characterize the underlying biological mechanisms associated with the disease, pathway and gene ontology enrichment analyses will be conducted.

**Conclusion:** Future studies combining transcriptomics with other omics strategies, including epigenomics, may provide helpful, comprehensive and specific biomarkers or targets to treat endometriosis. Through the identification of functionally significant alterations in gene expression in tissues involved in endometriosis, together with their associated molecular pathways available from reference databases, biomarkers for early and non-invasive diagnosis can be identified.

**Keywords:** Endometriosis, MICRO-ARRAY, R programming, Differentially expressed genes.

### P-131

#### **Pre- and post-natally exposure to *Foeniculum vulgare* and *Linum usitatissimum* alter modifying enzymes expression levels in NMRI mice ovaries**

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**Background:** Developmental exposure to exogenous estrogens, like phytoestrogens, may impact reproductive system development and its epigenetic signature.

**Objective:** This study investigated the impact of pre- and postnatal dietary exposure to phytoestrogen-rich fennel (*foeniculum vulgare*; FV) and flax (*linum usitatissimum*; FX) on ovarian epigenetics in first-generation offspring.

**Material and Methods:** In this experimental study, 32 NMRI pregnant mice (25-30 g, 6-8 wk old) were randomly divided into four groups (n = 8/ group): a control group receiving standard rodent chow, a fennel group (FV) receiving 500 mg/kg/day fennel, a flaxseed group (FX) receiving 500 mg/kg/day FX, and a combination group (FV + FX) receiving both. These treatments were administered via diet throughout

gestation and lactation until female offspring reached puberty. On postpartum day 56, the right ovaries of female offspring were harvested for quantitative real-time polymerase chain reaction analysis of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs).

**Results:** Ovarian DNA methyltransferases expression was reduced in all treatment groups compared to controls ( $p < 0.05$ ), with the FV group exhibiting the lowest levels ( $p = 0.03$ ). While FX had minimal impact on HDACs expression, the FV group showed significantly decreased HDAC1 and HDAC2 expression ( $p < 0.05$ ). Conversely, co-exposure to fennel and flax significantly reduced HDAC1 ( $p = 0.03$ ) and increased HDAC2 ( $p = 0.04$ ) expression.

**Conclusion:** Pre and post-natal exposure to a diet regimen supplemented with fennel or FX could induce ovarian epigenetic alterations in the F1 adult pups, potentially impacting germline development.

**Keywords:** *Foeniculum vulgare*, *Linum usitatissimum*, Ovary, Epigenetics, First generation.

**The original full text of this abstract has been published:**

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## **P-132 mRNA-RBP-miRNA network related to ferroptosis pathway in recurrent implantation failure**

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**Background:** Implantation is crucial in human reproduction, requiring effective communication between the embryo and the endometrium. Recurrent implantation failure (RIF) occurs when implantation does not succeed after multiple embryo transfers, impacting about 10% of couples undergoing in vitro fertilization. Factors influencing RIF include immunological, hematological, endocrine, and genetic aspects, particularly involving the ferroptosis pathway, a process of cell death linked to oxidative stress. Dysregulation of microRNAs (miRNAs) can also lead to reproductive disorders like RIF. Despite many studies on the role of non-coding RNA (ncRNA) and RNA-

binding proteins (RBPs) in disorders related to the reproductive system, the role of these ncRNA related to the ferroptosis pathway is unclear.

**Objective:** This study aimed to analyze the RBPs-miRNAs network involved in RIF and its role in the ferroptosis pathway.

**Materials and Methods:** This bioinformatic study utilizes a systems biology approach with various bioinformatics tools including: NCBI Gene Expression Omnibus (GEO), FerrDb v2 database, RBP database, miRDIP v5.3.0.2, STARbase, and Cytoscape 3.10.3 software, to identify key genes related to ferroptosis pathway and their potentially regulatory RBP-miRNA network associated with RIF. The mRNA transcriptome data was extracted from the GEO database using three RNA-seq datasets: GSE111974 and GSE71332. The first dataset (GSE111974) comprised 48 samples, (including 24 RIF samples and 24 fertile control samples). The second dataset (GSE71332) contained the miRNA expression profile of seven RIF patients and 5 control endometria. The FerrDb v2 database was used to extract a list of genes associated with the ferroptosis pathway. StarBase was employed to analyze mRNA-RBP (RNA-binding protein) interactions, while the miRDIP v5.3.0.2 online tool was utilized to establish a miRNA-RBP network. The RBPDB database served as a reference for the list of RBPs, and the RM2 target was applied to identify interactions between RBPs and RNA modifiers.

**Results:** This study utilized bioinformatics analysis to investigate the expression and significance of differentially expressed ferroptosis-related genes (DEFGRs) in RIF. We intersected a total of 1,388 ferroptosis-associated genes with 386 differentially expressed genes to identify key elements related to RIF. From this analysis, we identified 11 DEFGRs that showed differential expression between individuals with RIF and healthy controls. In subsequent analyses, we gathered 417 RBPs from the RBP database and the STARbase database, ultimately identifying 3 specific RBPs, *FUS*, *RBM47*, and *ZC3H7B*. We also identified 48 common microRNAs (miRNAs) from the GEO dataset, as well as those associated with the *FUS*, *RBM47*, and *ZC3H7B* via the miRDIP database. Finally, we constructed a network that links 11 mRNAs, 3 RBPs, 48 miRNAs, and 43 RNA modifiers using Cytoscape 3.10.3 software. These 3 RBPs including *FUS*, *RBM47* and *ZC3H7B* play an important role in RIF and infertility due to their interaction with their target genes in the ferroptosis pathway.

**Conclusion:** These findings highlight the importance of 3 RBPs including: *FUS*, *RBM47*, *ZC3H7B* in developing RIF and their role in the ferroptosis pathway, offering valuable insights into the molecular mechanisms involved in this condition. Further research into these networks may lead to innovative diagnostic and therapeutic strategies to address the ferroptosis pathway in reproductive health.

**Keywords:** mRNA, Recurrent implantation failure, DEFRG.

### P-133

#### Unveiling mitochondrial gene regulation in female infertility: Insights from non-coding RNA networks

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**Background:** Mitochondria are crucial for cellular energy production and homeostasis, playing an indispensable role in reproductive processes. Mitochondrial dysfunction has been closely linked to female infertility, manifesting in poor oocyte quality, diminished ovarian reserve, and impaired embryogenesis. This condition is multifactorial, arising from combination of genetic, epigenetic, and mitochondrial factors. Recent research has highlighted the importance of non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), as key regulators of mitochondrial gene expression and function.

**Objective:** In this study, bioinformatics approaches were employed to explore the lncRNA-miRNA network involved in mitochondrial pathways and its contribution to female infertility.

**Materials and Methods:** This bioinformatics-based study aims to elucidate the regulatory networks involved in female infertility. Genes associated with female infertility were identified using the GeneCards database, while mitochondrial genes were obtained from MitoCarta3. Intersection analysis revealed 140 genes (mRNA). To construct the mRNA-miRNA network, the miRWalk database was utilized, focusing on the "Genes" module along with the "5'UTR" and "3'UTR" platform, applying a score threshold of 0.95. By intersecting the identified miRNAs with those associated with infertility from the HMDD, a total of 10 key miRNAs were identified. The miRNet 2.0 database through the "ncRNAs" platform was utilized to investigate lncRNA-miRNA interactions. A network depicting the lncRNA-miRNA relationships associated with mitochondrial pathways was constructed and visualized using Cytoscape 3.10.3 software. The CytoHubba plugin was employed to identify key miRNA and lncRNA hubs with the highest interactions, applying the infertility clustering algorithm.

**Results:** The analysis highlighted 10 critical miRNAs hsa-miR-133b, hsa-miR-206, hsa-miR-378c, hsa-miR-449a, hsa-miR-543, hsa-miR-663b, hsa-miR-621, hsa-miR-4261, hsa-miR-1260a and hsa-miR-1302, associated with mitochondrial dysfunction in infertility. A total of 166 lncRNAs were identified as potential interactors with 5 miRNAs, hsa-miR-133b, hsa-miR-

206, hsa-miR-378c, hsa-miR-449a, and hsa-miR-543, using the miRNet 2.0 databases. We developed a network involving 5 miRNA and 25 lncRNA that is associated with mitochondrial functions, consisting of 50 nodes and 78 edges, utilizing cytoscape version 3.10.3. Our study revealed 5 top lncRNAs, *KCNQ1OT1*, *NEAT1*, *HCG18*, *SMIM25*, and *miR29B2CHG*, indicating significant interaction levels, with support from the CytoHubba plugin using Radiality parameter.

**Conclusion:** These findings underscore the significance of non-coding RNAs in the pathogenesis of female infertility, providing valuable insights into the molecular mechanisms underlying this condition. Certain miRNAs influence female infertility by affecting mitochondrial function and oxidative stress, crucial for oocyte quality. Further research into these networks may pave the way for innovative diagnostic and therapeutic strategies targeting mitochondrial dysfunction in reproductive health.

**Keywords:** Infertility, Mitochondria, miRNA, lncRNA.

### P-134

#### Gene expression of IL-33, IL-33 receptor and IL-37 in the endometrial tissues of women with endometriosis

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**Background:** Endometriosis as an inflammatory disease is characterized by the growth of endometrial tissue outside the uterine cavity. Although the exact pathogenesis of endometriosis remains unknown immune system dysfunction has been reported in this disease. Cytokines as soluble mediators of the immune and inflammatory responses, are known to be involved in the pathogenesis of endometriosis. The Interleukin1 family (*IL-1*) consists of several member, including *IL-33* and *IL-37*. *IL-37* has anti-inflammatory properties, while *IL-33* has inflammatory effects using *ST2* as its receptor. Several studies have shown the changes of these cytokines in the serum or peritoneal fluid of women with endometriosis.

**Objective:** This study aimed to investigate the gene expression of *IL-33*, *IL-33* receptor (*ST2*) and *IL-37* in the endometrial tissues of women with endometriosis compared to endometrial samples of control women.

**Materials and Methods:** In this ongoing case-control study, 15 women with endometriosis confirmed during

diagnostic laparoscopy as the endometriosis group and 15 women without endometriosis as the control group will be enrolled. Women aged between 20-45 yr old will be enrolled. Women who had any endometrial cellular changes benign uterine tumors or known inflammatory diseases will not be included in this study. After signing the informed consent, the endometrial tissues of the endometriosis group (eutopic and ectopic samples) and the endometrial tissues of the control group will be collected. Ectopic samples were obtained during laparoscopy while eutopic and control samples were collected by pipelle. After RNA extraction and cDNA synthesis, the relative expression of the studied genes (*IL-33*, *IL-33R* and *IL-37*) is performed using glyceraldehyde 3-phosphate dehydrogenase as a housekeeping gene and quantitative reverse transcriptase polymerase chain reaction.

**Results:** Till now, 13 women (5 women with endometriosis and 8 control women) have enrolled in this study. There was no significant differences in expression of these genes (*IL-33*, *IL-33R*, *IL-37*) in endometrial tissues of endometriosis group compared to control ones ( $p = 0.980$ ,  $p = 0.661$ ,  $p = 0.999$ , respectively).

**Conclusion:** According to this preliminary data, *IL-33* and *IL-37* gene expression did not differ between endometriotic and normal endometrial samples. Completion of the sample size is needed for conclusion.

**Keywords:** Endometriosis, Endometrium, *IL-33*, *ST2*, *IL-37*.

## P-135

### The effect of ovarian stimulation on peripheral blood natural killer T cells in endometriosis women undergoing assisted reproductive technology cycles

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**Background:** Endometriosis is a common disease of women in reproductive age characterized by the presence of endometrial glands or stroma cells outside the uterus. Endometriosis often leads to infertility and chronic pelvic pain. Among the various factors involved in its pathogenesis, immunological changes, including

alterations in immune cells such as natural killer T (NKT) cells have been reported in previous studies. On the other hand, using gonadotropin-releasing hormone agonists for the long-term is one of the therapeutic approaches of endometriosis but the effect of short-term use of these drugs during ovarian stimulation cycles has been less investigated.

**Objective:** This study aimed to investigate the effects of drugs used in ovarian stimulation cycles, on peripheral blood NKT cells (pNKT) in infertile women with endometriosis underwent assisted reproductive technology.

**Materials and Methods:** In this prospective cohort study which is conducted at the Royan infertility clinic, Tehran, Iran between 2024 and 2025, 40 infertile women diagnosed with endometriosis will be enrolled. Peripheral blood samples (3 mL) were obtained during the ovarian stimulation protocols on three times: the 2<sup>nd</sup> or 3<sup>rd</sup> day of the menstrual cycle, starting day of gonadotropins (if possible) and ovum pick-up day. Samples were stained with specific antibodies including peridinin chlorophyll protein anti-human CD3 (T cells marker), allophycocyanin anti-human CD56 (NK cells marker), fluorescein isothiocyanate (anti-human CD16 (another NK surface marker), and phycoerythrin anti-human CD107a (NK activity marker) antibodies. After staining, lysis buffer was added to each sample and phosphate-buffered saline was used for washing. The samples were analyzed using flow cytometry.

**Results:** Thus far, 23 women with a mean age of 33.17 yr old and a mean body mass index of 24.72 kg/m<sup>2</sup> have been included in the study. A comparison of blood samples between 2 time points (2<sup>nd</sup> or 3<sup>rd</sup> days of the menstrual cycle and ovum pick-up day) revealed that the frequency of pNKT cells (CD3+CD56+) showed a non-significant decrease ( $p = 0.4864$ ). Additionally, cytotoxicity markers on CD3+ cells (CD3+CD16+CD107a+) did not significantly changed ( $p = 0.1696$ ).

**Conclusion:** At the current sample size, no significant changes in pNKT cells or their cytotoxicity have been observed. Completing the sample size of this study is needed to provide a clear understanding of the effects of ovarian stimulation drugs on pNKT cells.

**Keywords:** Endometriosis, Natural killer T cells, Gonadotropin releasing hormone agonist, Gonadotropin releasing hormone antagonist, ART, Peripheral blood.

## P-136

### New combinational immunotherapy with co-administration of PDL-1 siRNA and bemcentinib in the cervical cancer cell

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**Background:** Potential immunotherapy cancer microenvironment modulators have shown lasting cancer treatment effects. New anticancer drugs targeting programmed cell death-ligand 1 (PD-L1) are immune checkpoint blockers (ICBs). ICB blockers cannot antagonize all resistance pathways, hence ICB-based immunotherapy benefits a restricted number of cancer patients. Cancer patients have a bad prognosis due to the multistep epithelial-to-mesenchymal transition (EMT) that caused metastasis. AXL receptor tyrosine kinase and its signaling enhance cancer metastasis and chemoresistance through the EMT process and alter immune response in the tumor microenvironment, making them crucial to cancer carcinogenesis. Previous studies link AXL expression to ICB-based immunotherapy resistance in tumor models and cancer patients. Thus, creating combination treatments with synergistic mechanisms or overcoming ICB-based resistance is necessary. In addition to antibody-based treatments, gene silencing by small interfering RNAs (siRNAs) has recently been investigated to modify the cancer milieu to boost the immune response.

**Objective:** Our experimental study aimed to explore new combinatorial immunotherapy by targeting PDL-1 and AXL using siRNA and bemcentinib, respectively, in cervical cancer cells.

**Materials and Methods:** Firstly, the transfection efficiency of siRNA in PDL-1 knockdown and IC50 of bemcentinib as a tyrosine kinase inhibitor were determined at the time point. Additionally, the basal relative expressions of related genes were assessed. Cervical cancer cells, Caski, were transfected with PDL-1 siRNA (10 nM) using lipofectamine RNAiMAX. After 6 hr of incubation, cells were treated with 2.5  $\mu$ M bemcentinib. The following groups were appointed: scramble si, PDL-1 si, scramble si + bemcentinib, PDL-1 si + bemcentinib, bemcentinib, and non-treated cells. Relative PDL-1 and AXL, transforming growth factor beta, vimentin, and matrix metalloproteinase 9 mRNA expression, known as EMT-regulating genes, were measured by quantitative real-time polymerase chain reaction. The MTT assay was used to evaluate the viability of Caski cells after co-administration of PDL-1 siRNA and bemcentinib in related groups.

**Results:** Caski cells expressed an acceptable level of related genes. It was also shown that PDL-1 siRNA significantly inhibited PDL-1 mRNA expression levels in a time-dependent manner (24 and 48 hr) ( $p < 0.0001$ ). Our results revealed combining PDL-1 siRNA with bemcentinib dramatically reduced mRNA expression of PDL-1, transforming growth factor-beta, vimentin, and matrix metalloproteinase 9 but not AXL in PDL-1 si + bemcentinib vs. scramble si + bemcentinib ( $p < 0.0001$ ). Also, the MTT assay showed that the knockdown of

PDL-1 along with AXL inhibition led to a significant reduction of viability in caski cells in targeted groups ( $p < 0.02$ ).

**Conclusion:** The results of this study present new horizons in neoadjuvant cancer immunotherapy which render overcoming chemoresistance and broaden the scope of cancer patients who benefit from ICB-based immunotherapy. However, further in vivo investigations are necessary.

**Keywords:** Cervical cancer, Combinational immunotherapy, PDL-1 siRNA, Axl receptor tyrosine kinase.

## P-137

### Post-weaning exposure to sunset yellow FCF induces alterations in apoptosis signaling pathway in rat testicular tissue: Modulating effects of coenzyme Q10

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**Background:** Research on the harmful health effects of azo dyes remains limited and yields inconsistent findings. Coenzyme Q10 (CoQ10) is known for its antioxidant and anti-inflammatory properties, which may offer protective benefits across various organ systems.

**Objective:** This study aimed to evaluate the potential toxicological impact of the commonly used food dye Sunset Yellow (SY) on the apoptotic signaling pathways in rat testicular tissue and to assess whether CoQ10 supplementation could mitigate these effects.

**Materials and Methods:** 60 Sprague-Dawley male weanling rats (45-50 gr, 21 days old) were randomly divided into 6 groups ( $n = 10$ /each). The rats received their treatments via daily oral gavages for 6 wk. The groups received: low-dose SY (2.5 mg/kg/day), high-dose SY (70 mg/kg/day), CoQ10 (10 mg/kg/day), CoQ10 combined with low-dose SY, CoQ10 combined with high-dose SY, or distilled water (control). At the end of the treatment period, animals were euthanized, and testicular tissues were collected for gene expression analysis via quantitative real-time polymerase chain reaction and histopathological examination using hematoxylin and eosin staining.

**Results:** High-dose SY significantly downregulated *BCL2* expression ( $p = 0.035$ ) and upregulated *BAX* ( $p < 0.001$ ) and *CASP3* ( $p = 0.006$ ) genes compared to controls. Testicular weight ( $p = 0.029$ ), volume ( $p = 0.014$ ), and serum testosterone levels were significantly reduced in the high-dose group. CoQ10 co-treatment

provided partial protection against these alterations but did not fully reverse them.

**Conclusion:** High-dose SY induces testicular toxicity by disrupting apoptosis-related gene expression and testicular function. CoQ10 offers partial protective effects, though not sufficient to fully counteract SY-induced damage.

**Keywords:** Food additive, Apoptosis, Coenzyme Q10, Testis, Caspase.

### P-138

#### Study the effect of platelet-rich plasma on gene related stem cells genesis expression in ovarian failure mouse model

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**Background:** As a novel therapeutic approach, platelet-rich plasma (PRP) has the potential to revitalize ovarian tissue in women with premature ovarian failure (POF). Octamer-binding transcription factor 4 (OCT4), a POU domain transcription factor, is essential for early embryonic development and is predominantly expressed in stem cells. This transcription factor is expressed in stem cells, suggesting a fundamental role in the establishment of pluripotency.

**Objective:** This study utilized 8-10-wk-old (25-30 gr) Syrian mice to investigate the effects of PRP on POF.

**Materials and Methods:** A cyclophosphamide-induced POF model was established in female mice. The mice were randomly divided into three groups (n = 7/each): 1) control group, 2) POF + PRP, 3) PRP group. After injecting a dose of PRP ( $7 \times 10^7$  platelets), the mice were given 2 wk, then the mice were sacrificed and the ovaries were removed to evaluate the expression of the *OCT-4* gene in different groups.

**Results:** The expression levels of the *OCT-4* gene were observed to be significantly elevated in the groups that received PRP treatment compared to the POF group, with a statistical significance threshold of ( $p < 0.001$ ).

This indicates a significantly increase in *OCT-4* gene expression associated with the application of PRP.

**Conclusion:** Given the significant increase in gene expression associated with stem cell genesis under PRP intervention in a mouse model of ovarian failure and the use of more extensive studies, PRP can be proposed as a treatment for restoring ovarian potency.

**Keywords:** POF, Stem cells, Gene expression, PRP.

### P-139

#### New insights into cervical cancer screening: The role of DNA methylation testing in prognosis and triage

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Cervical cancer is regarded as one of the most prevalent cancers in women and continues to provide a substantial public health challenge, particularly in resource-limited settings. Traditional screening and monitoring techniques for cervical cancer, such as the pap smear and high-risk human papillomavirus (hrHPV) test, have markedly diminished the worldwide incidence of cervical cancer. However, these methods cannot reliably distinguish between transient lesions and those that progress to precancerous or cancerous states. This results in difficulties in patient management, including overtreatment of lesions or overlooking some precancerous cases (undertreatment). Studies find that hypermethylation of tumor suppressor genes is markedly associated with several malignancies, including cervical cancer. An important aspect during the process of cervix carcinogenesis is the link between chronic hrHPV infection and epigenetic changes (especially DNA methylation) on certain host cell genes. Viruses like hrHPVs reprogram the epigenetic landscape of the host cell genes involved in immune evasion, cell cycle control, and apoptosis, ensuring the virus's presence in the cell and causing chronic infection. During chronic infection and with the accumulation of epigenetic changes, the progression favors the development of malignant lesions and invasive cervical cancer. Recent research indicates the effectiveness of DNA methylation testing as a complementary method for the triage and monitoring of cervical cancer. DNA methylation is an epigenetic modification that usually causes gene silencing without altering the DNA sequence. In cervical cancer, abnormal DNA methylation is observed in almost all malignancies and is strongly associated with persistent hrHPV infection. Understanding the underlying mechanisms of cancerous lesion formation and the relationship between methylation patterns and chronic hrHPV infection can

help in identifying the risk of cancer and consequently in better management and treatment of cervical cancer. Research shows that nearly all cervical cancers have hypermethylation, making these patterns crucial for early detection and risk evaluation. Incorporating DNA methylation analysis into routine screening may enhance the specificity and sensitivity of existing hrHPV tests. The review highlights the correlation between DNA methylation patterns and disease progression as well as patient outcomes. Methylation-driven indicators are correlated with survival rates in cervical cancer patients, providing new opportunities for personalized therapy approaches. The detection of high-risk lesions via methylation analysis may enable prompt therapies and enhance overall survival rates. This review emphasizes the necessity for ongoing study into the function of epigenetic alterations in cervical carcinogenesis and their prospective use in clinical practice, with the goal of alleviating the burden of this preventable disease by novel screening measures. As we go towards personalized healthcare, DNA methylation testing has potential for increasing screening effectiveness and strengthening prognostic precision and patient outcomes in the management of cervical cancer.

**Keywords:** Cervical cancer screening, DNA methylation, Human papillomavirus, Triage.

#### P-140

### Gene expression of *IL-18*, its receptors and *IL-18* binding protein in the endometrial tissues of women with endometriosis

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**Background:** Endometriosis as a chronic inflammatory disease affects women of reproductive age and characterized by the growth of endometrial-like tissue outside the uterus. Endometriosis can cause chronic pelvic pain and infertility. Several studies have shown that one of the etiologic factors in the pathogenesis of endometriosis is a dysregulation of immune responses and alteration of different cytokines as immune cells production is reported in women with endometriosis. Interleukin 18 (*IL-18*) a member of the (*IL-1*) family has proinflammatory effects. *IL-18* binding protein (*IL-18BP*) is the natural inhibitor of *IL-18* that its signaling

is mediated through the *IL-18* receptor complex (*IL-18R1*, *IL-18RAP*). Different studies showed that alterations of the *IL-18* system are involved in the pathological process of endometriosis.

**Objective:** This study aimed to evaluate the gene expression levels of *IL-18*, its receptors (*IL-18R1*, *IL-18RAP*) and *IL-18BP* in the endometrial tissue of women with endometriosis.

**Materials and Methods:** In this ongoing case-control study, 30 women who underwent diagnostic laparoscopy will be enrolled at Royan institute, Tehran, Iran between 2024 and 2025 (15 women with endometriosis as the endometriosis group and 15 women without endometriosis as the control group). Inclusion criteria consist of age between 20-45, regular menstrual cycles and not receiving hormonal drugs in the past 3 months. Women who had malignant endometrial cellular changes or benign uterine masses were excluded. Ectopic endometrial samples from endometriosis patients were collected during laparoscopy while eutopic endometrial samples were collected simultaneously with a pipelle, as well as endometrial samples of the control group were obtained using a pipelle. After tissue samples collection, RNA extraction is performed using Kiazole, and then cDNA synthesis was performed using SMOBio Technology kit. Gene expression levels were examined using real time polymerase chain reaction. Glyceraldehyde-3-phosphate dehydrogenase was used as housekeeping gene.

**Results:** Till now, 5 women with endometriosis and 8 control women were enrolled. 10 tissues of endometriosis group (5 ectopic samples, 5 eutopic endometrial samples) and 8 normal endometrial tissues (control) were studied. There was no significant differences in gene expression of *IL-18*, *IL-18BP*, *IL-18R1* and *IL-18RAP* between endometriosis and control groups ( $p = 0.0653$ ,  $0.6971$ ,  $0.1419$ , and  $0.2084$ , respectively).

**Conclusion:** According to this preliminary data, no significant differences was found in gene expression of *IL-18* and its related genes (*IL-18BP*, *IL-18R1*, *IL-18RAP*) between endometriotic and normal endometrial tissues. Definite conclusion will be made after the enrollment of 30 women and completion of the study.

**Keywords:** Endometriosis, *IL-18*, *IL-18* receptors, *IL-18* binding protein, Inflammation, Gene expression.

#### P-141

### Seminal cell-free nucleic acids as possible biomarker in male infertility: A mini-review article

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Male infertility, accounting for nearly 40% of infertility cases worldwide, is a growing clinical concern with multifactorial etiologies, including genetic, environmental, lifestyle, inflammatory, and age-related factors. Despite available diagnostic tools such as semen analysis, endocrine and genetic evaluations, and invasive procedures like testicular biopsy, the underlying causes of idiopathic infertility remain unclear in many cases. Recent studies have highlighted the potential of seminal cell-free DNA (cfDNA) as a promising non-invasive biomarker for assessing male fertility. cfDNA, originally identified in human plasma in 1948, is released through mechanisms like apoptosis, necrosis, or cellular lysis and can be found in various body fluids, including seminal plasma. Elevated levels of cfDNA in semen have been correlated with impaired sperm morphology and motility, suggesting its association with oxidative stress, inflammation, and reproductive dysfunction. Factors such as obesity, exposure to environmental toxins, high scrotal temperature, radiation, and exogenous androgens can increase reactive oxygen species and disrupt DNA integrity in sperm. Moreover, aging contributes to DNA damage, telomere shortening, and decreased antioxidant defenses, all of which negatively impact spermatogenesis. Genetic anomalies, including Y chromosome microdeletions (especially in the AZF region), mutations, and polymorphisms, are also implicated in male infertility, emphasizing the need for advanced genomic tools in clinical diagnostics. Inflammation within the male reproductive tract often asymptomatic can further exacerbate infertility through the release of pro-inflammatory cytokines detectable in semen. Given the current limitations of conventional diagnostic methods, cfDNA profiling offers a promising avenue for understanding the molecular basis of male infertility, improving diagnostic accuracy, and optimizing assisted reproductive technologies. This review consolidates recent findings on seminal cfDNA, underscoring its diagnostic value and the need for further research to validate its clinical applications.

**Keywords:** Male infertility, Cell-free DNA, Cell-free nucleic acid, Seminal plasma, Serum.

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## P-142

### Alteration of interleukin-35 levels in seminal plasma and male serum of couples with recurrent spontaneous abortion

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**Background:** Recurrent spontaneous abortion (RSA) as one of pregnancy complications is defined as the loss of  $\geq 2$  pregnancies before 24 wk of gestation. Although several factors including parental chromosomal translocations, uterine anomalies and several maternal conditions are involved in RSA pathogenesis, but no known cause could be found in some RSA cases known as idiopathic RSA (iRSA). Emerging evidence suggests that maternal immune response towards the semi-allogenic embryo plays important role in some cases of iRSA. On the other hand, seminal plasma, as surrounding fluid of sperm plays a role in nourishing and protecting the sperm. It also contains signaling molecules such as cytokines that affect reproductive tract. It was shown that an optimal balance between immunomodulatory and pro-inflammatory cytokines in seminal plasma are essential for normal embryo implantation and placentation. Interleukin (IL) 35, as new member of IL-12 family, has anti-inflammatory properties and yet the role of this cytokine does not been studied in male partner of iRSA couples.

**Objective:** The aim of this study was to evaluate IL-35 levels in seminal plasma and male serum of couples suffering from iRSA.

**Materials and Methods:** In this case-control study, male partners of couples with iRSA group and fertile men who had one or more child and referred to Royan institute, Tehran, Iran between November 2023 to July 2024, Tehran Iran for sex selection of next child (control group), were enrolled. Men with genital tract infection, hypogonadism, history of chemotherapy, venereal diseases or using any immune-modifying medications were not enrolled. Semen samples were collected by masturbation after 48-72 hr of sexual abstinence and blood samples were collected at same day. Blood samples were centrifuged at 3500 rpm for 10 min. Beyond liquefaction of semen, semen samples were

centrifuged for 10 min at 6000 rpm to remove sperm and cellular debris. The concentration of IL-35 was detected in serum and seminal plasma using an enzyme-linked immunosorbent assay kit.

**Results:** 23 male partners of iRSA group and 21 fertile men were enrolled. The mean age of control group was significantly higher than iRSA group ( $39.45 \pm 3.80$  vs.  $37.13 \pm 3.53$ ,  $p = 0.045$ ). No significant differences were found between 2 groups in regard to body mass index, smoking and alcohol consumption. The mean seminal levels of IL-35 was significantly higher in iRSA group than control group ( $6.16 \pm 0.99$  vs.  $5.11 \pm 1.05$  ng/ml,  $p = 0.001$ ). However, no significant difference was observed in regard to IL-35 levels in blood serum.

**Conclusion:** This study showed that seminal levels of IL-35, were significantly higher in male partners of iRSA group compared to fertile men. Previous studies suggested that decreased levels of pro-inflammatory cytokines and increased levels of anti-inflammatory cytokines in seminal plasma led to defective invasion and, consequently, abnormal placentation leading to recurrent miscarriages. Further studies on other cytokines in seminal plasma are recommended.

**Keywords:** Interleukin-35, Seminal plasma, Blood serum, Recurrent spontaneous abortion, cytokine.

### P-143

#### Therapeutic potential of stem cell-derived exosomes in infertility disorders

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Infertility disorders affect many individuals worldwide, with recent lifestyle changes contributing to its increasing prevalence in both males and females. Therefore, discovering innovative approaches to prevent and treat infertility is of critical importance. Stem cells offer many advantages in regenerative medicine and are considered an ideal source for exosome extraction. Exosomes have the ability to penetrate barriers and easily deliver therapeutic cargo to target cells. Due to their low immunogenicity, reduced tumorigenic risk, and anti-apoptotic and anti-inflammatory properties, they have emerged as a promising tool in fertility preservation, diagnosis, treatment, and the prevention of infertility and pregnancy-related disorders. This narrative review aims to explore the clinical applications and advancements made by stem cell-derived exosomes

in male and female infertility. A search was conducted on PubMed and Google Scholar for English articles related to the clinical applications of stem cell-derived exosomes in infertility and reproductive diseases, with a publication date range from 2020-2024. A total of 25 articles were retrieved. Based on relevance, focus on infertility-related conditions, publication type, duplication, and accessibility, 10 articles were included in the final review. The selected studies focused on infertility and related conditions including premature ovarian failure, polycystic ovarian syndrome, preeclampsia, endometriosis, intrauterine adhesion, asthenozoospermia, and azoospermia. Reported outcomes included enhanced follicular development, angiogenesis, and ovarian granulosa cell growth; improved ovarian environment and endometrial thickness; restoration of spermatogenesis and testicular function; increased sperm quantity, motility, and morphology; and reduced oxidative stress. Overall, with superior biological stability and lower immunogenicity compared to stem cells, exosomes have the potential to overcome some of the challenges associated with cell-based therapies. The high therapeutic potential of exosomes highlights the importance of thoroughly investigating the underlying mechanisms of action to enable their clinical translation in the future.

**Keywords:** Stem cell, Exosome, Infertility, Reproductive disorders.

### P-144

#### Study of consanguinity in women with premature ovarian failure in Yazd Infertility Center in 2024

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**Background:** In recent years, as the age of marriage increases, premature ovarian failure is diagnosed at a time when treatment is difficult and impossible. Early diagnosis can prevent complications and problems of this disease and give hope to these patients' lives.

**Objective:** Given the high prevalence of consanguineous marriage in Iran and Yazd province, this study was designed to investigate the role of genetic kinship and family history of the disease in the occurrence of premature ovarian failure.

**Materials and Methods:** This is a retrospective descriptive study. The statistical population consisted of 31 women with premature ovarian failure who referred to the Yazd Reproductive Sciences Institute, Yazd, Iran. The inclusion criteria for patients were a definitive diagnosis of premature ovarian failure and access to family information and marital status of these individuals. The exclusion criteria for patients were unavailability of case information and premature

ovarian failure with secondary causes such as chemotherapy or ovarian surgery. First, we used Excel software to record and categorize the data. Data including age of onset of the disease, type of marriage, relationship to the spouse, and history of the disease in first and second degree relatives were extracted. We use *t* test and one sample proportion test for statistical analysis of data.

**Results:** Statistical analysis showed that there is a statistically significant relationship between consanguineous marriage and the prevalence of premature ovarian failure. The relationship between family history and premature ovarian failure was also significant. The parents of 17 out of these 31 women had

consanguineous marriages, and among these 17 cases, 6 mothers of individuals with premature ovarian failure had a history of miscarriage.

**Conclusion:** The results of this study showed that there is a statistically significant relationship between consanguineous marriage and family history of the disease with the occurrence of premature ovarian failure. According to the results of the study, it is recommended that pre-pregnancy counseling be performed in families with a history of this disease, and that awareness and screening be performed in areas with a high percentage of consanguineous marriage.

**Keywords:** *Premature ovarian failure, Infertility, Consanguinity.*