ABSTRACT BOOK

ROYAN 2020

International Twin Congress

Reproductive Biomedicine & Stem Cells

September 2- 6, 2020 Tehran- Iran

21st September 2 - 4, 2020
Congress on
Reproductive Biomedicine

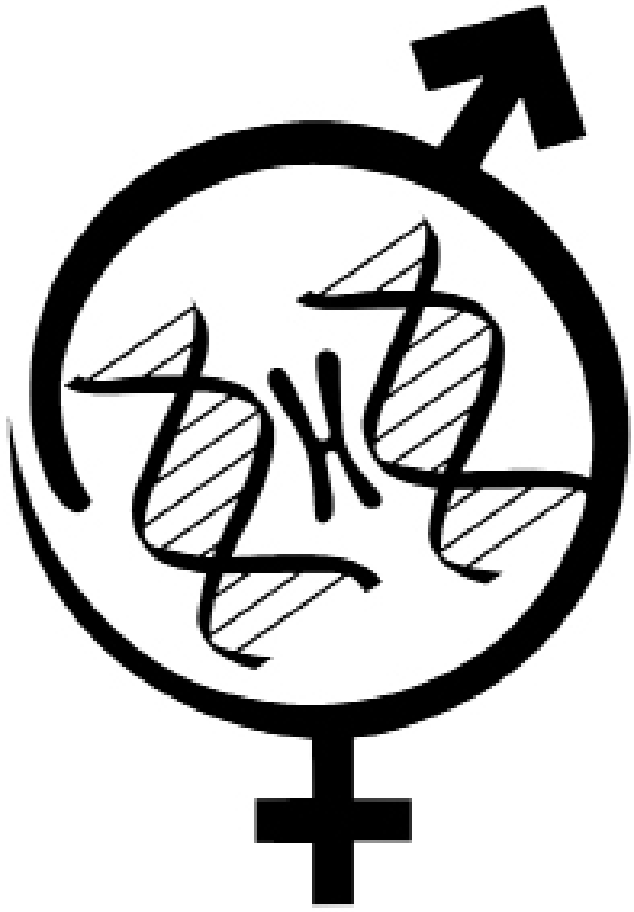
16th September 5 - 6, 2020
Congress on
Stem Cell Biology & Technology

Abstracts of

Royan International Virtual Twin Congress

21st Virtual Congress on Reproductive Biomedicine
2-4 September 2020

16th Virtual Seminar on Nursing and Midwifery
3 September 2020



Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Abstracts of the
21st Congress on Reproductive Biomedicine
16th Seminar on Nursing and Midwifery

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Congress Chairperson

Firouzeh Ahmadi

On behalf of the Organizing Committee, it is my pleasure to invite you to 21st Royan Virtual International Congress on Reproductive Biomedicine (2-4 September 2020), Tehran, Iran.
Thanks to the leading role of Royan Institute in Reproductive Biomedicine and Stem Cells, since its establishment by the late Kazemi Ashtiani in 1991; Royan International Congress has been designed to provide an innovative and comprehensive overview of the latest research developments in both fields, primarily in the areas of Endocrinology, Gynecology, Andrology, Genetics, Imaging, Epidemiology, and Embryology.
Royan Institute has succeeded in holding 20 International Congresses and welcomed more than 100 world-class distinguished scholars and researchers of the fields to present and share the latest breakthroughs in the field. The scope and quality of the scientific exchange have evolved through these years to address the clinical as well as research-based achievements that turned this event into a particularly prominent scientific event in the Middle East and the World. Since then, Royan Congress has paved the way for novel paths of joint-research collaboration and partnership with prominent global institutes and scientific congresses of the fields (namely MEFS and Karolinska).
Due to the COVID19 pandemic across the world, we have decided to convert the congress to a fully virtual event, this year. In the upcoming 21st Royan virtual congress, just like the previous congresses, the scientific program is scheduled to include keynote speakers, plenary sessions, and other programs such as educational and viable workshops.
On behalf of the scientific committee, it is my sincere pleasure to invite the distinguished researchers to join us as invited speakers. The organizing committee intends to provide a wonderful forum for you to meet, interact and exchange your ideas with the Iranian and International outstanding scientists

Yours sincerely,
Firouzeh Ahmadi, PhD
Chairperson of the 21st Royan
Virtual Congress on Reproductive Biomedicine

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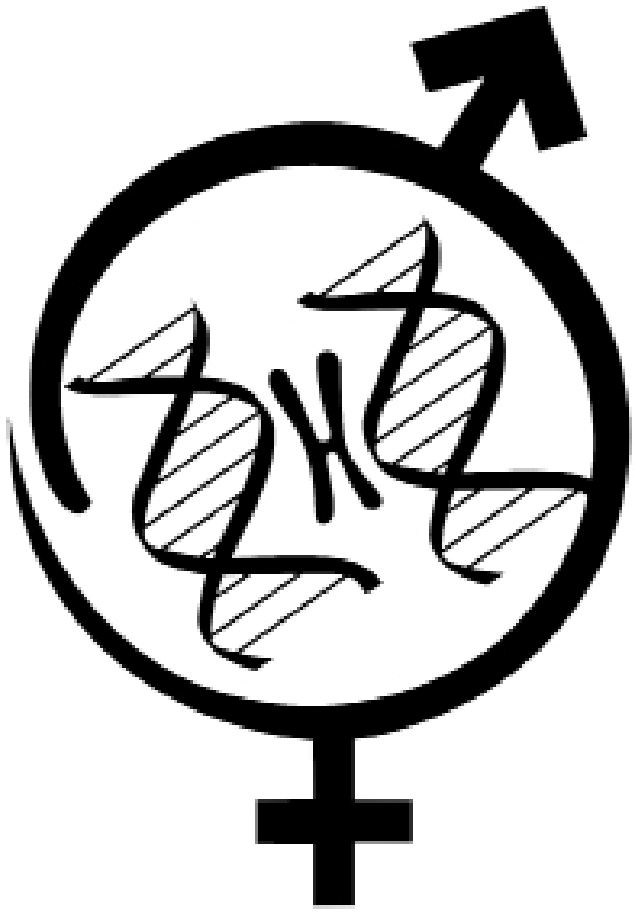

Royan Institute

Reproductive Biomedicine Research Center
Tehran, Islamic Republic of Iran

Andrology

I-1: The Male Contribution to Infertility and The Health of Future Generations

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Fertility rates are falling rapidly across the globe with the result that a majority of countries, including Australia and Iran, are now at or below replacement rate and ultimately facing population decline. The reasons for this infertility pandemic are complex and include a range of social, economic, political and biological factors. The purpose of this lecture is to highlight the importance of male infertility in this overall equation. Something significant and sinister is certainly happening to the male reproductive system: the last half century has seen a global increase in the incidence of testicular cancer, such that it has risen from relative obscurity to become the most common cancer in young males; developmental disorders such as cryptorchidism are also riding rapidly; sperm counts have halved and male infertility is at an all time high. Recent data also suggest that the male germ line is responsible for 75% of all de novo mutations in our species and is responsible for a number of genetic diseases particularly the rising tide of brain disorders (including autism, spontaneous schizophrenia and bipolar disease) and childhood cancer. In this lecture I shall present data to indicate that oxidative stress is a dominant feature of male infertility, induced by a variety of clinical and environmental factors from age and varicocele to smoking and obesity. Oxidative stress is also central to mutagenesis in the male germ line. Within the spermatozoon a region of chromosome 15 appears to be particularly vulnerable to oxidative attack and this genetic locus also appears to be strongly associated with male mediated pathology in the offspring including brain disorders, cancer and infertility. Strategies are now needed to reduce, and ultimately prevent, this stress form occurring and, importantly in an assisted reproductive technology (ART) context, to select spermatozoa lacking this form of damage.

I-2: Medical Treatment of Male Infertility: Medical Treatment of Hypogonadotropic Hypogonadism Men

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Hypogonadotropic hypogonadism (HH) is defined as a syndrome due to problems with either the hypothalamus or pituitary gland that result to gonadal failure. HH is approximately 1-10:100,000 live births, and approximately 2/3 of cases are caused by Kallmann syndrome. HH is characterized by androgen deficiency and absent/delayed or arrested pubertal sexual maturation and infertility. Although diagnosis is mainly based on history and physical examination but other anterior pituitary hormones including prolactin, thyroid-stimulating hormone, insulin-like growth factor 1 and cortisol had to be measured to exclude multiple defects, and magnetic resonance imaging undertaken if appropriate.
In adult men who wish to be father, human chorionic gonadotropin (hCG) at a starting dose of 1500 IU s.c. twice weekly is used. Some patients require greater stimulation by doses of up to 10,000 IU twice weekly to generate normal testosterone levels. Treatment with hCG alone occasionally may result in semen production in those with larger pre-treatment testes (>8 cc) and no history of cryptorchidism.
If severe oligospermia or azoospermia persists after 3-4 months of hCG, 75-150 IU of recombinant human FSH s.c. or i.m. three times weekly is indicated.
Combination of hCG and follicle -stimulating hormone (FSH) therapy for 6-24 months results in testicular growth in almost all patients and spermatogenesis in 80-95%.
Factors predicting successful outcomes include larger baseline testicular size and absence of cryptorchidism, prior history of sexual maturation and no prior androgen therapy.
Although there is some evidence that GnRH therapy may stimulate testicular growth at a faster rate than gonadotropins, most studies have shown no advantage of gonadotropin - releasins hormone (GnRH) over gonadotropin therapy.

I-3: Different Endocrine Treatments of The Infertile Male: Indications, Limitations and Results: Treatment of Hypogonadotropic Hypogonadism

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Whereas 15 % of couples in the reproductive age are known to suffer from difficulties in initiating a pregnancy, it is estimated that male factors are at least partially responsible for this challenge in half of the cases. Since the invention of ICSI, unfortunately the careful evaluation and specific treatment of the male has developed very slowly and several centers have adopted the practice of solving the male issue by offering ICSI treatment to couples without properly investigating the etiology of the decreased male fertility. It is clear that the most severe cases of male factor infertility often require testicular biopsy combined with ICSI fertilization, however, there are clear cases (e.g hypogonadotropic-hypogonadism) where the semen quality and hence fertility can be improved by appropriate hormonal treatments. In addition, in many cases optimizing the male fertility prior to fertility treatments is simple and effective, even if the significant improvement cannot be guaranteed to all.
In order to identify the men, who benefit from hormonal treatments it is of paramount importance to understand the regulation of testicular function, which will enable to differentiate primary and secondary problems in the testicular function.
Hypogonadotropic-hypogonadism (e.g. Kallman syndrome) – typically puberty has been induced by hormonal treatment, a sophisticated pediatric endocrinologist may have initiated the treatment by FSH alone, however, more typically the induction has been carried out by testosterone. Most hypo-hypo men will respond to induction of spermatogenesis and reach normal or near normal sperm counts with a long enough gonadotrophin

treatment. Typically, hCG is used at 2500-5000 iu ×2/week, it is important to check that the testosterone response is sufficient (testosterone replacement therapy is obviously discontinued). If sperm do not appear in semen following a treatment of 6 mos, FSH is combined to the treatment (150-300 iu x 3 / week). Patience is required as it may take 1-2 years before sperm appear. Not all will reach normospermia.
Normo-hypogonadism (normal LH but low -lowish testosterone) – despite the low testosterone level the pituitary secretion of LH is inappropriate. This finding is often associated with obesity (probably due to fat-tissue originated estrogen suppression). LH secretion can be improved by aromatase inhibitor (e.g. letrozole) or antiestrogen (e.g. tamoxifen). These oral medications will rapidly increase LH secretion as well as testosterone (T) levels, this should be checked after 2-3 weeks of treatment. As the maturing of sperm is fairly slow, the improvement in semen quality will only be apparent after 4 mos. Further improvement may take place with continued treatment. Not all men respond to this treatment, if after an appropriate response in LH and T levels no improvement is apparent at 4 mos the treatment is unlikely to be beneficial. hCG may subscribed instead, typically 2500-500 iu 2×/week. Adjusting the estrogen metabolism/effect may cause side-effects, decreased libido is the most common one.
The use of anabolic steroids has clearly increased also among non-professional athletes. As all anabolic steroids have androgenic effects, they also suppress gonadotrophin secretion from the pituitary and decrease sperm production, typically azoospermia. This effect is reversible (e.i. spermatogenesis will recover with discontinuation), however, often the symptoms of hypogonadism are severe and active treatment is required. Aromatase inhibitors as well as antiestrogens may be used to stimulate gonadotrophin secretion from the pituitary. Sometimes hCG is more effective. The principle is to assist the recovery of the pituitary – gonadal -axis from the typically severe suppression caused by androgenic treatments. The same principles can be used when fertility is requested during androgen replacement therapy for late-onset-hypogonadism (andropause).
Disturbances in prolactin secretion and thyroid dysfunction need to be treated appropriately.

I-4: Antibiotic Therapy in Male Infertility

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The effect of infection on male infertility is controversial. Although the bacterial, viral and protozoa infection of male genitourinary tract is important and could be an etiology of male factor infertility but still there is some debate on this matter. Infection could deteriorate the spermatogenesis, impair sperm function and could cause obstruction of seminal tract. The detection of infection is difficult because of contamination and effect of seminal plasma. Antibiotic therapy could in some cases promote the sperm parameters and increase the rate of pregnancy but not in all cases. The effect of leukospermia is also controversial. The relation between sperm DNA fragmentation and leukospermia should be considered in evaluation of male infertility.

Animal Biotechnology

I-5: An Electrotransfection Protocol for Efficient Gene Editing in Mammalian and Avian Cells

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Development of an electrotransfection protocol for efficient deletion and insertion in the target genomic DNA is highly demanded for plasmids expressing the CRISPR/Cas system. In this study, we developed a square-wave pulsing protocol based on OptiMEM-GlutaMAX medium for highly efficient transfection of murine embryonic fibroblasts (EF), induced pluripotency stem (iPS), farm animal, and avian fibroblast cells using both reporter genes and gRNA/Cas9-encoding plasmids. An electrotransfection efficiency of > 95% was achieved using reporter-encoding plasmids in both mouse EF and iPS cells, as well as bovine, ovine and chicken EF cells. Up to a 98 % targeted gene knockout was implemented by indels (insertion/deletion) producing by gRNA/Cas9 plasmids in transgenic cells carrying a single-copy of a fluorescent Venus reporter. Targeted deletions were also efficiently carried out in the Venus gene of murine cells (up to 67 % deletion rate) as well as BMP15, GDF9, and ACTR1B endogenous genes of ovine and avian EF cells by co-electroporation of two gRNA-encoding plasmids. Then, using gRNAs targeting the promoter region of Venus transgene, in which NHEJ did not induce a Venus knockout, we established a screening platform in which only homology-directed repair (HDR) with the donor plasmid could abrogate the functionality of the Venus transgene. Based on this detection system, we obtained up to 12 % targeted HDR rate via electrotransfection of large plasmid donors (12.5 to 15.5 kb) into nocodazole-treated mouse EF cells. In summary, we introduced a plasmid electrotransfection protocol which is straight-forward and efficient for CRISPRing mammalian and avian primary cells.

I-6: Genetic Engineering to Enhance Livestock Genotypes

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The development of technologies for the direct modification of livestock genomes has made it possible to engineer livestock with enhanced characteristics. Technological progress has seen a tremendous shift from random additions of genes by transgenic approaches to genome editing with the ability to efficiently and precisely introduce changes in livestock genomes almost without limitations. The presentation will provide examples to illustrate the application of different approaches to enhance livestock genomes for biomedical and agricultural purposes.

I-7: Superovulation Induces Autophagy in Placenta of Day 15.5 Concepti in Mice

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Assisted reproductive techniques (ARTs) have been linked to a wide range of pregnancy disorders such as intra uterine growth restriction (IUGR), pre-term delivery, low birth weight (LBW) and preeclampsia (PE). Most research about these disorders has focused on the embryo itself and little attention has been paid to possible effects on the placenta. In this study, we evaluated the effect of superovulation during pre- and post-implantation development on autophagy related genes in 15.5 day placenta in mouse. Regard to this five experimental groups were designed to determine the effect of superovulation during pre- and post-implantation development: control (C), control/embryo transfer/natural synchronized (CTC), superovulation (S), superovulation/embryo transfer/natural synchronized (STC), superovulation/embryo transfer/superovulated synchronized (STS). According to our results, the weight of the fetus and also the ration of the fetus weight to the placenta weight were decreased in S, STC and STS groups compared with C and CTC groups. Autophagy is an inducible catabolic process activated by external stressors such as nutrients starvation and hypoxia. Here we explored, whether autophagy and apoptosis were involved in the abnormal placentation caused by superovulation. The higher mRNA expression of autophagy-related markers including *Beclin1*, *Atg5*, *Atg7*, and *Lc3b* in addition to higher expression of ATG7 and LC3BII/LC3B1 in protein level showed a disordered autophagy in placentas under conditions of superovulation exclusively during post-implantation development (S and STS) compared with C, CTC and STC groups. These data suggest that superovulation can impair autophagy in placenta following superovulation. However, the signal pathway by which superovulation causes aberrant autophagy needs to be explored further.

Embryology

I-8: Management of IVF Laboratories during Covid-19 Pandemic

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The sudden and unpredictable onset of COVID-19 epidemics requires preparation programs for embryology / andrology laboratories at infertility treatment centers, as well as protocols to ensure the safety of gametes / embryos and laboratory personnel.
Reproductive societies around the world, as well as the Iranian Society of Reproductive Medicine (ISRM), had recommendations for fertility clinics to completely or drastically reduce clinical practice, leading to a change in the management of IVF laboratories in three stages: complete shutdown, maintenance and finally restart.
Preparation involves a lot of pre-epidemic programs and preparations, and now with this experience, we are going to be prepared for similar situations. The plan includes logistical arrangements, reducing labor needs, saving equipment, safeguards for embryologists and biological materials.

I-9: The New Procedure of Oocyte Vitrification: Advantages and Disadvantages

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Vitrification has been the latest bigger improvement in the IVF field, not only for the laboratory, providing a stable and superior rate of survival; but also, for the clinicians, as new medical protocols are now possible, as the freeze-all strategy.
This talk is focused in the most complex cell to cryoconserve, the oocyte, due to its high content of water, and its importance in the success of the whole IVF treatment.
Oocyte vitrification provides new options for women willing to postpone their fertility, but also a solution for cancer patients, and on patients without a valid sperm sample on the day of pick-up.
Its main advantages and disadvantages will be described, but also, technical tricks to share with another embryologist.

I-10: Covid 19 and Management of Cryoperservation in Assisted Reproductive Technology

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Female Infertility

I-11: The Effect of Alloimmune Factors and Immunotherapy in RIF and Idiopathic Recurrent Abortion

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Pregnancy is considered as a semi-allograft as fetus expresses paternal antigens. Immunological dysregulation is considered as one of the important factors involved in repeated implantation failure (RIF) and recurrent spontaneous abortion (RSA). Different immunological factors and immune cells such as cytokines, growth factors, dendritic cells, macrophages, decidual and uterine NK cells, as well as different T helper cells have been considered as the causes of RIF and RSA. Different therapeutics agents including aspirin, immunosuppressive drugs, intravenous immunoglobulins (IVIG), hydroxychloroquine and intrauterine infusion of different agents such as PRP, PBMC, G-CSF and hCG are suggested in treatment of RIF.

I-12: Oxidative Damage in Male Infertility: Can We Really Fix It?

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It is widely accepted that sperm DNA fragmentation is involved in male infertility and that it is the result of oxidative damages occurring during sperm maturation. It was therefore postulated that such oxidative aggression is caused by an oxidative imbalance, i.e. oxidative stress, and that a treatment with antioxidant substances, i.e. oral antioxidant preparations, might address the problem. However, in spite of more than 20 years of clinical investigations there is not yet any proof that oral antioxidants improve male fertility and evidences of no efficacy or even of detrimental effects are accumulating. Indeed, while their ability to balance the oxy-redox system is questionable, antioxidants may interfere with sperm nuclear maturation. Promising results have been recorded with micronutrients intended to stimulate the activity of the endogenous antioxidant system within the control of homeostatic regulations, which also improves the nuclear maturation, but clinical confirmations are as well lacking. On the other side, it is now understood that reactive oxygen species are mainly generated in mitochondria and that this happens when mitochondria are in reductive imbalance, which is unlikely to be addressed by any antioxidant intervention. Accordingly, to implement affective intervention, we need to better understand the sperm oxy-redox physiology. Meantime, aggressive antioxidant treatments should be avoided.

I-13: Challenges of Surgical Intervention and Optimizing Oncologic and Reproductive Outcomes

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Ovarian cancer is the seventh most common cancer among women with a high rate mortality. In 2018, 4.4% of entire cancer-related mortality among women was attributed to ovarian cancer (OC).
Despite usually occurring in older patients, 3%–14% of all OC are diagnosed at youngers with a very high rate of overall 5-year survival (91.2%) in women ≤44 years of age if diagnosed at very early stage. As all standard therapies for OC, including surgical intervention and chemotherapy, may be harmful for the ovaries, fertility sparing surgery (FSS) should be discussed with premenopausal women with early-stage OC who desire to have children, before any treatment planning.
Many studies have investigated the aspects of oncologic and reproductive outcomes after FSS, and todays, the ART available to cancer patients and survivors is developing.
The standard surgery for OC includes a TAH and BSO with comprehensive staging in early stages or debulking in advanced stages based on the histology of the disease. Most common histologic type of OC is epithelial which has 4 main histologic subtypes (serous, endometrioid, mucinous and clear cell). Less common ovarian histopathology (LCOH), include carcinosarcoma, clear cell carcinoma, mucinous carcinoma, low grade serous/endometrioid epithelial carcinoma, borderline epithelial tumors, malignant sex cord stromal tumors, and malignant germ cell tumors.
In selected young patients who wishes to preserve their fertility, FSS (preserving the uterus and contralateral ovary) should be offered. Based on published studies and guidelines, FSS could be considered if the intraoperative exploration and frozen section of the ovarian mass show: malignant germ cell tumors, borderline epithelial tumors, clinical stage I epithelial ovarian tumors, clinical stage I mucinous tumors and clinical stage I sex cord stromal tumors.
For considering the FSS; surgeon and patient must carefully discuss potential risks and benefits before the surgery.
Overall, in selecting appropriate candidates for FSS, survival outcomes do not appear to differ between FSS and radical surgery and is best done through MDT including (gynecologic oncologists and fertility specialists). Besides; preconception counseling with perinatologist is also important to optimize health before a woman attempts to conceive.

I-14: Fertility Preservation Strategies in Ovarian Tumors

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Ovarian cancers make up 2.6 % of all female cancers .12.1% of ovarian cancer patients are ≤44 years of age. 1.5% of all consultations on fertility preservation were initiated after the diagnosis of an ovarian tumour. There is a harmful effect of surgery and chemotherapy and radiation therapy on fertility. Current fertility-preservation methods are fertility-sparing surgical approaches , ovarian tissue cryopreservation , oocyte and embryo cryopreservation , protection against germ cell damage using fertoprotective agents, Invitro ovarian follicle maturation. Fertility-sparing surgery only appears to be sensible and oncologically safe for women under 40 years of age , with a desire to have children , who have unilateral FIGO IA G1 ovarian cancer or borderline ovarian tumours.After fertility sparing surgery in Borderline ovarian tumors the best option is to achieve pregnancy through spontaneous conception immediately after first surgery.In situations involving a personal history of infertility or when reproduction is not desired by patients yet, the most appropriate plan is to rely on assisted reproductive techniques , with oocyte harvesting and cryopreservation after fertility sparing surgery. *In vitro* studies have not shown a proliferative effect of FSH or estradiol on primary cultures of BOT .The concomitant use of letrozole to reduce estrogen levels during COH is a potentially useful strategy, but strong evidence about is lacking.In epithelial ovarian cancers cryopreservation of embryos or oocytes after the performance of fertility sparing surgery is recommended. COH is not indicated in patients with granulosa cancer due to rapid hormone-dependent proliferation. Carcinosarcomas and Malignant Mixed Müllerian Tumours are rare tumours with a poor prognosis. They are not candidates for fertility-sparing surgery .Immature oocytes could be acquired during unilateral ovariectomy and matured in vitro either before freezing or after thawing.In ovarian cancer patients, transvaginal oocyte retrieval carries a risk of ovarian capsule rupture and cancer cell spillage, which can cause staging up from 1A to 1C.Oocytes can be retrieved from the unaffected ovary during surgery, with or without COH.Up to now, data are lacking on ovarian cancer relapse rates after gonadotropic stimulation .Ovarian cryopreservation not recommended in patients with ovarian cancer . ovarian tissue may be a therapeutic strategy if, instead of autotransplantation of the tissue, the still experimental techniques for the generation of oocytes by xenotransplantation in other species, in vitro maturation or formation of an artificiortal ovary .fertoprotective agents adjuvants consist of

:sphingosine-1-phosphate (S1P), imatinib mesylate,amifostin, tamoxifen and GnRH antagonists and agonists , melatonin. Ovarian suppression using GnRH analogs (GnRHa) to prevent ovarian reserve from chemotherapy-induced damage has been controversial due to conflicting result.

I-15: Pre-IVM: Variation on IVM

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I-16: The Epidemiology of Coronavirus in Iran

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I-17: The Effect of Hereditary and Non-Hereditary Thrombophilia in RIF and Recurrent Abortion

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Thrombophilia are hereditary and/or acquired conditions that predispose patients to thrombosis. The association between thrombophilia and recurrent pregnancy loss (RPL) has become an undisputed fact. Thrombophilia creates a hypercoaguable state which leads to arterial and/or venous thrombosis at the site of implantation or in the placental blood vessels. Recurrent miscarriage (RM) is defined as the loss of three or more consecutive and clinically-recognised pregnancies before 20 weeks' gestation; this affects 1–2% of women. This incidence increases to 5% when it is defined as a loss of two or more clinically-recognised pregnancies before 20 weeks' gestation. Thrombophilia is a common cause of RPL and may be seen in 40–50% of cases. Pregnancy is a hypercoaguable state and if the pregnancy is affected by thrombophilia, the hypercoaguable state becomes worse and may impair blood flow through the maternal veins, leading to deep vein thrombosis, and clots in the placental blood vessels, leading to fetal growth restriction and/ or fetal demise. Due to this fact, anticoagulants have become very popular for treating RPL.
Who do we screen? Studies suggests that all patients with a history of prior venous thrombotic events and those with adverse pregnancy events such as fetal loss, abortions, RIF, severe intrauterine growth restriction and early onset severe preeclampsia, should be evaluated for thrombophilias.
In acquired thrombophilia, antiphospholipid syndrome (APS) can be due to either lupus anticoagulant antibodies or anticardiolipin antibodies, as seen in women with SLE. In APS, the body's immune system recognizes the phospholipids, which are a part of the cell membrane, as a foreign substance and thus produces antibodies against them. However, other studies have shown that antiphospholipid antibodies (aPL) often act against a protein cofactor called β2- glycoprotein.
In inherited or genetic thrombophilia, there is usually a family history of excessive clotting. More commonly, the diagnosis is based on the demonstration of a gene mutation such as a Factor V Leiden (FVL) mutation MTHFR (C677T) MTHFR (1298A), a hyperhomocysteinaemia mutation (A506G), a prothrombin mutation (G20210A) or prothrombin II (PTII) mutation, FXIII Val34 Leu polymorphism or a protein S and/or C deficiency. High frequency of Val34Leu polymorphism in RM/RIF presumably speaks in favor of a multifactorial RM genesis, where an altered thrombophilia status plays a role.
Currently, many clinicians treat RPL—either associated with all types of thrombophilia or unexplained—with low-molecular-weight heparin combined with low-dose aspirin.This treatment became popular in the late 1990s, after Sanson et al. reported that thrombophilia is associated with the high risk of fetal loss in early and late pregnancy. Thrombophilia is either inherited, acquired or a combination of both.
Inherited thrombophilia is more prevalent in women with recurrent IVF failure compared with healthy women. Thrombophilia has a significant role in IVF–embryo transfer implantation failure. Women with repeated IVF–embryo transfer failure should be screened for thrombophilia. Anticoagulants are an effective treatment against RPL in women with acquired thrombophilia due to antiphospholipid syndrome. In women with RPL and APS, LMWH can be used as early as six weeks' gestation until 34–36 weeks' gestation.

I-18: Testicular sperm extraction and High sperm DFI

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Semen analysis , once considered as a corner stone in andrology clinic has been supplanted with sperm DNA fragmentation index (DFI) in specific condition. Increased sperm DFI may lead to decrease natural pregnancy rate and ART outcome. The role of testicular sperm extraction in patients with increased sperm DFI is the subject of debate. Testicular sperm may have better DNA integrity in comparison with their ejaculated counterpart, but there is controversy about the rate of their chromosomal aneuploidy and the health of the off spring. In addition testicular sperm extraction may have considerable complications. Therefor it is logical to reserve testicular sperm extraction for the patients with recurrent ejaculated-IVSI failure and persistent increased sperm DFI despite alternative therapy.

I-19: Fertlity Presrvation in Endomerioma

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Prevalence of endometriosis among infertile women is 25 to 50%. Thirty to fifty per cent of women with endometriosis are infertile.
The most common symptoms of endometriosis are dysmenorrhea, dyspareunia, chronic pelvic pain and/ or infertility. However some women may be asymptomatic.

Half of women affected by severe form of endometriosis are unable to conceive naturally.
Endometriosis itself and its surgical treatment can impair future fertility, so fertility preservation can be of help in this issue.
Medical therapy and its related suppressive effect does not improve conception rate and the only result is to delay for more effective treatment to conceive.
Endometriosis by itself can cause follicular damage even without previous surgical interventions
Repeated excisional and ablative procedures, which are commonly used to treat the endometriosis at the functional and active part of ovarian tissue is another problem concerning the endometriosis and infertility.
Increasing risk of malignancy which is suggested by a growing body of evidence could be another considerable issue for treating severe form of endometriosis.
As mentioned, endometriosis related infertility has both pathologic and iatrogenic causes, and fertility preservation is problem solving and should be considered at multiple levels as follow...
A. Surgery should be performed by an expert surgeon to minimize iatrogenic injury to the ovaries.
B. Oocyte freezing
C. Embryo freezing
D. Ovarian tissue cryopreservation
E. *In Vitro* maturation of oocytes and cryo preservation.

I-20: Reproductive Microbiota in A New Enigma

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Commensal bacteria are in the intestine, nasal and oral cavities, skin and urogenital tract, And they provide: defense against pathogens, shape development and maturation of immune system, digest food and fiber, produce vitamins, metabolize xenobiotics (Puebla 2014) Lactobacillus are dominant microbiota of lower and upper genital tract (Ravel 2011). Dysbiosis is changes in homeostasis of vaginal microbiota, and causes infertility and preterm labor and endometriosis. Vaginal microbiota and endometrial microbiota have direct relation to infertility and ART and lactobacillus dominancy is the key point. In cases of discharge and smell and itching and other symptoms, infertility clinician must evaluate patients for dysbiosis .

I-21: Implantation Failure: The Relevance of The Embryo vs other Factors

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The true limiting factor for a successful IVF cycle is the implantation of the embryo into the endometrium. This process, as we understand it today, is quite inefficient in humans whereas significantly more efficient in other species. One of the main factors contributing to this inefficiency is the high rate of aneuploid embryos that are generated even in fertile couples, bringing fecundability, or the chance of becoming spontaneously pregnant per month around 25%.
In IVF, we will be presenting data showing that after three single euploid blastocyst transfer, cumulative pregnancy rate is beyond 90%. The problem is obtaining 3 euploid embryos in the couples we treat, as most of them have poor ovarian reserve or advanced maternal age with a great majority of embryos being abnormal.
But not everything is aneuploidy. We will show that even after euploid embryo transfer, there will be more early pregnancy losses when comparing women under 35 vs women older than 35. So there must be other factors that contribute to a lower outcome.
We will briefly touch upon ultrasound evaluation of the uterus and adenomyosis, the role of routine hysteroscopy and endometrial scratching, endometrial transcriptomics as we understand it today, the intrauterine immune environment, and finally immunology of the endometrium in a stimulated cycle.

I-22: AMH Is The Real Biomarker of Ovarian Reserve

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Genetics

I-23: Non-Invasive PGT: Now and Future

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Preimplantation genetic testing (PGT) evolution for aneuploidy screening (PGS or PGT-A) and diagnosis of monogenic diseases (PGD or PGT-M) is dependent on developing embryo cell biopsy that could be harmful and needs highly skilled embryologists with extra-charges. Non-invasive PGT (niPGT) is an evolving field that uses spent embryo culture media (SCM) and/or blastocoel fluid (BF) instead of biopsied cells. niPGT is promised to be a revolution in reproductive genetics however we have to be realistic about advantages and disadvantages of niPGT state of the ART.
SCM is almost non-invasive while BF could be performed by lower levels of skills compared to biopsy. BF contains less DNA with higher risk of insufficient amplification, certainly allele drop out during PGT-M, in less sensitive DNA detection technologies. SCM harbor more DNA with higher risk of DNA contamination from cumulus cells, polar bodies, sperm cells, manufacturing processes, microbes and personnel. Overall ploidy and per chromosome discrepancies with biopsies and/or whole embryo in niPGTA is a common concern in both SCM and BF that might rises from used protocols before, in and after sample preparation, sample quality and DNA fragmentation due to necrosis and apoptosis, mosaicism and aneuploidy depletion. Higher concordance is expected in samples with meiotic errors, like advanced maternal age, those lead to more acceptable ART outcome in routine PGT-A. Considering healthy babies after transfer of some mosaic embryos, our

interpretation from relatively incident aneuploidies in SCM and BF might need to changes through randomized clinical trials. niPGT is still in preclinical stage and solving the aforementioned disadvantages should be considered before clinical application.

I-24: Controversies in Aneuploidy Screening: A Mosaic of Problems

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I-25: PGT-SR – The Oft Forgotten Third Wheel

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I-26: Expanded Carrier Screening Preimplantation Genetic Testing (PGT) for Adult-Onset Disorders

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Preimplantation genetic testing (PGT) for single gene disorders is now indicated for adult onset conditions. In 2001, our group at RGI began to perform PGT for heritable cancers in which neoplasia occurred throughout life. Many heritable cancers are characterized by varied expressivity and age of onset. Increasing numbers of PGT cycles are now performed for adult onset cancer, heart disease and neurodegenerative disease.
In our unit, 7,000 of the approximately 22,000 PGT cycles we performed are for single gene disorders. Over 1000 are for heritable adult onset disorders, 642 for heritable cancers. Typically, one partner was known to have a mutation but not yet manifesting. Desire existed to avoid affected offspring. Among 30 different heritable cancers, the most common indication was breast cancer due to mutations in BRCA 1 or 2. The most common BRCA 1 mutation was 187delAG, present in half of the BRCA1 mutations tested. The most common mutation in BRCA 2 was 6174delT, present in 40% of BRCA2 mutations tested. Linkage analysis is necessary to avoid diagnostic errors due to allele drop out (ADO). Our strategy requires study of affected family members and family members free of genetic predisposition to determine the haplotype of alleles linked to the mutant allele versus haplotype of alleles linked to the normal allele.
With concomitant PGT-M and PGT-Aneuploidy, liveborn rates after trophectoderm biopsy are increased 15-20% over pregnancy rates with PGT-M alone. No known diagnostic errors occurred among 642 heritable cancers to date.
PGT for adult-onset heritable cancers is diagnostically accurate, and results in robust livebirth rates.

Systematic Disorders, Obesity and Infertility

I-27: The Effect of Lifestyle Interventions on Fertility Treatment Outcome

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Infertility is the inability for a couple to achieve pregnancy within one year of regular and unprotected intercourse, and it has been identified as a major medical and social concern. Globally, an estimated 15% of couples in reproductive age are facing fertility challenges. Epidemiological studies have also shed light on a link between infertility and lifestyle factors. Data on the association of lifestyle factors with fertility and healthy reproduction have been accumulating. The established efficacy in fertility lifestyle programs has not been translated into better outcomes. Therefore, the purpose of this paper was to assess the effect of lifestyle interventions on fertility treatment.
There is a growing interest in the worldwide for modification of lifestyle factors to treat infertility. The most common modifiable lifestyle factors (e.g., body weight, dietary patterns, alcohol, tobacco, coffee, drugs, physical activity, stress and sleep) have been identified as risk factors for infertility disorders in both genders. Infertile couples, attending for fertility treatments are often advised to control their weights to improve the fertility outcomes. Nutritional status and dietary patterns, as major lifestyle factors, are crucial determinants of normal reproductive function. Nevertheless, adherence to a healthy lifestyle may improve fertility but making a lifestyle change is challenging due notably to behavioral factors. According to the recent meta-analysis in 2020, lifestyle interventions in obese infertile women that encompass behavioral modifications of physical activity and dietary intake probably contribute to make little difference in the improvement of live birth and a slightly increase in the pregnancy rate compared with the usual care group. Furthermore, these findings suggested a link between these lifestyle modifications and a slightly increase of the risk of miscarriage. There is a complete lack of appropriate long-term lifestyle interventions that could correlate changes in diet and other lifestyle factors with improvements of fertility. Hence, future clinical trials in this filed are needed. Overall, a stage-based intervention that modified quality of life of infertile couples before the start of assisted reproductive technology treatment may be effective to improve fertility in both males and females. These interventions should be designed based on patient needs, including elements on weight, diet, physical activity, alcohol and drugs, and psychological health.

I-28: The Effect of Obesity Surgery in Fertility Treatment Outcome

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Obesity is a common problem among women of reproductive

age. Obesity and overweight involves an abnormal and excessive fat accumulation that negatively affects the health status. Obesity effect mechanism is not fully known. However, it is supposed that obesity can cause infertility via various mechanisms. It is also supposed that weight loss, even a small amount can facilitate fertility. In individuals who are unable to lose weight with diet and lifestyle changes, bariatric surgery can be considered as an option. Among all the techniques sleeve gastrectomy and bypass are the most effective nowadays, because of their positive properties. The two techniques facilitate greater weight loss and have better outcomes or resolution of clinical conditions such as PCOS, type 2 diabetes mellitus, cardiovascular disease, and risk factors including the metabolic syndrome, glycemic control, hypertension, and dyslipidemia, sleep apnea, mortality, quality of life, and fertility.

I-29: Obesity and Infertility in Men and Women

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Obesity is increasing globally in men and women, and the negative impact of overweight and obesity on reproductive health, fertility, pregnancy outcomes, is significant. Both male and female fertility are impacted on by being overweight or obese. Obesity impairs both natural and assisted conception. Although the pathophysiology is not clear, it appears that obesity impacts endocrine function in men and women, oocyte and sperm quality, embryo quality, endocrine receptivity, and implantation. Miscarriage, pregnancy, and live birth rates and the risk of congenital malformations are all influenced by obesity.
Menstrual irregularities occur more frequently in women who are obese. This is due to a functional alteration to the hypothalamic-pituitary-ovarian (HPO) axis from various factors. Additionally, adipokines, leptin, tumor necrosis factor alpha (TNFα), and interleukins produced from adipose tissue, are known to impact on ovulation and HPO axis.
Like in the female, the hypothalamic-pituitary-gonadal (HPG) axis is dysregulated in the setting of male obesity. There is strong evidence of a negative effect of obesity on total testosterone, sex hormone binding globulin (SHBG), and free testosterone as well as reduced inhibin B concentrations and diminished luteinizing hormone (LH) pulse amplitude. It is well understood that suppression of SHBG by hyperinsulinemia in obese men increases androgen availability for aromatization to estrogen in adipose tissue, which may then lead to negative feedback and reduction in gonadotrophin secretion. Consequent to this is a decreased Leydig cell testosterone secretion, which ultimately affects spermatogenesis. The impact of male obesity on sperm parameters in humans is more controversial, with many contradicting studies. Studies have also confirmed that male obesity is associated with higher levels of sperm DNA damage, due to the oxygen-free radical damage, and a direct thermal effect on the testicles due to obesity.
Preconceptional weight loss is recommended for all women and men seeking fertility treatment, firstly through counseling, lifestyle intervention, and behavior modification and then with adjunctive pharmacological agents or bariatric surgery, Careful consideration of the benefits of delaying conception for weight loss must be balanced against the possibility of declining fertility due to advancing age of the couple.

I-30: Obesity and Sexual Dysfunction in Men and Women

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Obesity, one of the major growing problems of the present century is reaching pandemic proportions. Sexual problems in both sexes appear to be widespread in society and are associated with impaired quality of life. Obesity may impair sexual function through multiple mechanisms. Obesity, has a major role in the development of vascular defects in the reproductive system, resulting sexual dysfunction especially in men. The association between obesity and increased insulin resistance has also led to molecular changes in the vasculature, especially in the context of reduced bioavailability of nitric oxide as a vasodilator, leading to dysfunction of the penis and clitoris, resulting sexual dysfunction in men and lack of orgasm in women. In women, obesity may induce/exacerbate polycystic ovary syndrome and induce unpleasant changes in the appearance of the person such as acne and hair loss in women, affecting the body image and resulting in sexual dysfunction. The impact of obesity on sexual dysfunction from the Immunological, endocrinological, psychogenic, and anatomical aspects will be review in this presentation.

Andrology

P-1: The Effect of Paroxetine on Sperm Parameters in Mice

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Background: In the 21th century, one of the most relevant diseases which world is involved in it is Major Depression Disorders (MDDs). Up to now, a plethora of anti- depression drugs have designed such as Paroxetine, Fluoxetine, Sertraline, etc. Paroxetine is known as Selective Serotonin Reuptake Inhibitor (SSRI) that most physicians prescribe it to patients involve in depression. However, anti-depression drugs have several side-effects on health in contrast to the advantages. Thereby, our study was the assessment of sperm parameters in mice received Paroxetine.
Materials and Methods: We selected twelve mice that divided them to two group including control (CT) (n=6) and paroxetine (PAX) (n=6) groups. CT and PAX groups was administrated with water (500µl)/daily and Paroxetine (7 mg/kg)/ daily during sixty days, respectively. Two months later, the mice were sacrificed and the caudal epididymis were extracted and sperm parameters such as sperm total motility (and also in further detail: sperm progressive, non-progressive and immotile of spermatozoa), concentration and abnormal defective morphology, were assessed. All of the statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS Inc., Version 25.0) and an independent student t-test was used for comparison of variations between two groups. $P<0.05$ was considered statistically significant.
Results: The mean sperm concentration (million/ml) ($P<0.05$) and the percentage of sperm total motility ($P<0.05$), sperm motility, sperm progressive motility ($P<0.05$) and non-progressive motility ($P<0.05$) decreased significantly in mice received PAX compared to CT group, while the mean percentage of immotile sperm assessment showed the increase in PAX group in comparison to CT group ($P<0.05$). Furthermore, the mean percentage of spermatozoa pop defective forms increased in PAX group against to CT group ($P<0.05$).
Conclusion: Our results demonstrated that anti-drugs depression could have side-effect on sperm production process if the healthy individuals consumed it.
Keywords: Depression, Sperm Parameters, Sperm Form, Paroxetine, SSRI

P-2: Evaluation of The Activity of SOD in Testis Tissue of Cholestasis Rats

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Background: Cholestasis causes severe damages, such as oxidative stress in the human body. In males, testicles are also affected, and inflammation and oxidative stress are markedly induced in these organs. SOD is regarded as the most well-known antioxidant enzyme in Leydig and Sertoli cells of the testis. In this study, we tried to measure the activity of SOD in testicular tissue of cholestasis rats.
Materials and Methods: Eight adult male Wistar rats were divided into two groups named as control and cholestatic (4 each). To induce obstructive cholestasis their common bile duct was closed by surgery. The activity of the SOD enzyme was quantified in control and cholestasis groups by means of the SOD enzyme assay kit according to the manufacturer's instructions.
Results: Our findings demonstrated that the activity of SOD was significantly decreased in the cholestasis group compared with the control group; so that the activity of this enzyme in the control group was 754.2 ± 26.48 U/ml, whereas in the patient group it was reduced to 506.3 ± 44.41 U/ml ($P= 0.003$).
Conclusion: Previous studies have identified a correlation between seminal SOD with abnormal sperm morphology, percentage of dead spermatozoa, and sperm count in infertile males. In the current study, it has been shown that SOD level was declined in testicular tissues of the cholestasis group in comparison with healthy rats.
Keywords: Cholestasis, SOD, Spermatogenesis, Oxidative stress, Testis

P-3: The Study of Co-Administration of The Ophylline and Zinc on Hormonal Changes in Men with Asthenoteratozoospermia: A Double-Blind Clinical Trial Study

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Background: Theophylline is recently used to improve viable sperm quality for assisted reproductive technology (ART). Zinc as a strong antioxidant is commonly prescribed in treatment of male subfertility. We investigated the effect of co-administration of theophylline and zinc on hormonal changes in men with asthenoteratozoospermia.
Materials and Methods: 22–55 year-old subfertile men with asthenoteratozoospermia participated in this study and were divided into four groups. All of the groups were randomly assigned to receive one of four treatments for 12 weeks: Placebo, theophylline (200 mg/day), zinc (220 mg/day) and theophylline + zinc. The serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were measured by immunoradiometric assay with riakey kits, Korea at the beginning and the end of treatment. Data was analyzed statistically using the repeated measurements ANOVA and the means were considered significantly different at $P<0.05$.
Results: The serum testosterone level increased significantly in all three groups compared to the placebo group but this increase was significantly higher in the theophylline + zinc group than the theophylline and zinc groups. The serum LH level increased significantly in all three groups compared to the placebo group but this increase was significantly higher in the theophylline group than the theophylline + zinc and zinc groups. The serum FSH level increased significantly in all three groups compared to the placebo group but this increase was significantly higher in the zinc group than the theophylline + zinc and theophylline groups.

Conclusion: Co-administration of theophylline and zinc could modify the changes in the hormonal profile of asthenoteratozoospermic men. Therefore, this drug combination can be prescribed in cases of infertile men with asthenoteratozoospermia.
Keywords: Theophylline, Zinc Sulfate, Male Infertility, Hormonal Changes

P-4: The Outcome of Sperm Chromatin Integrity, Viability and Reactive Oxygen Species Level in Male Infertility before and after Theophylline and Zinc Sulfate Co-Effect

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Background: Theophylline is a methyl-xanthine derivative that has antioxidant effect and influences the sperm parameters. Zinc sulfate has been investigated as an antioxidant supplementation for stimulating spermatozoa. We aimed to evaluate the possible effects of the co-administration of theophylline and zinc sulfate on sperm chromatin integrity, viability and reactive oxygen species (ROS) level in subfertile men.
Materials and Methods: Patients participated in this study were referred to Rastak Infertility Treatment Center, Sina Hospital for infertility treatment. They were divided into four groups: Placebo, theophylline, theophylline + zinc sulfate and zinc sulfate. The sperm viability and sperm chromatin integrity were analyzed using the Eosin-Nigrosine and Aniline blue staining respectively and the sperm ROS level was analyzed using Chemiluminescence assay before and after three months of oral treatment. Data was analyzed statistically using the repeated measurements ANOVA and the means were considered significantly different at $P<0.05$.
Results: Sperm chromatin integrity and viability increased significantly in all three groups compared to the placebo group but this increase was significantly higher in the theophylline + zinc sulfate group than the theophylline and zinc sulfate groups. Sperm ROS level increased significantly in the theophylline group, while it showed a significant reduction in the zinc sulfate group compared to the placebo. There was no significant difference in the mean sperm ROS level in the theophylline + zinc sulfate group compared to the placebo.
Conclusion: Theophylline and zinc sulfate, as two antioxidants, could improve the sperm chromatin integrity and viability. Zinc sulfate could reduce the increasing sperm ROS level that is from the side effects of theophylline.
Keywords: Theophylline, Zinc Sulfate, Viability, Maturity, ROS

P-5: Evaluation of Spermogram and Gene Expression Changes in Infertile Men after Theophylline and Zinc Sulfate Co-Administration

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Background: Theophylline elevates cAMP dependent reactions such as sperm motility,however it can also damage sperm DNA integrity. Moreover, zinc sulfate can prevent sperm DNA damages. We investigated the co-administration of theophylline and zinc sulfate on the Bcl-2, Bax and Caspase-3 genes expression in infertile men with asthenoteratozoospermia.
Materials and Methods: 120 asthenoteratozoospermic patients were randomly divided to 4 groups: Placebo, theophylline, theophylline + zinc sulfate and zinc sulfate. After 90 days of oral treatment, the sperm parameters were analyzed using the CASA system and the expression of genes was also analyzed using Real-Time PCR at the beginning and the end of trial. Data was analyzed statistically using the Repeated measurements ANOVA and the means were considered significantly different at $P<0.05$.
Results: Sperm motility, count and morphology increased significantly in all three groups compared to the placebo group but this increase was significantly higher in the theophylline + zinc sulfate group than the theophylline and zinc sulfate groups. The expression of Bcl-2 gene increased significantly in the zinc sulfate group, while it showed a significant reduction in the theophylline group compared to the placebo. The expression of Bax and Caspase-3 genes increased significantly in the theophylline group, while it showed a significant reduction in the zinc sulfate group compared to the placebo. There was no significant difference in the expression levels of Bcl-2, Bax and Caspase-3 genes in the theophylline + zinc sulfate group compared to the placebo.
Conclusion: The theophylline and zinc sulfate co-administration could improve the sperm parameters and protect the sperm DNA from the undesirable effects of theophylline.
Keywords: Theophylline, Zinc Sulfate, Asthenoteratozoospermia, Sperm Parameters, Gene Eexpression

P-6: Peroxiredoxin Acts as A Novel Inhibitor to Ferroptosis

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Background: Ferroptosis is a necrotic cell death that arises from iron overload and indicated by oxidative damage to phospholipids. Ferroptosis initiated depending on the lipid peroxidation process that activated by enzymatic mechanism (lipoxygenases) and non-enzymatic (Fenton reactions). To date, ferroptosis has been documented to be regulated only by glutathione peroxidase 4 enzyme and radical-trapping antioxidants. However, peroxiredoxin is another effective enzyme that acts to inhibit lipid peroxidation, but it was not previously considered in explaining the mechanism of ferroptosis. Peroxiredoxin has been recognized to represent 1% or more of total cellular proteins in animals, and its activity was been found to be responsible for the dissociation of more than 90% of mitochondrial peroxides and 99% of cytosolic peroxides in humans. Peroxiredoxin is an important antioxidant enzyme reported to have a role in male fertility and sperm function. The current study was designed to investigate the correlation between the peroxiredoxin enzyme and ferroptosis via measuring the qualitative and quantitative properties of seminal fluid, in parallel with the oxidant and antioxidant balance in the semen of patients with asthenozoospermia.
Materials and Methods: This study included 120 subfertile

male partners from couples who had consulted the infertility clinic of Kerbela Hospital of Maternity (Kerbela governorate, Kerbela city, Iraq) between July 2019 and July 2020. A complete medical history was recorded and physical examination was completed for each. Peroxiredoxin activity, Glutathione peroxidase activity, Glutathione peroxidase 4 concentration, lipid peroxidation, total antioxidant and total reactive oxygen species were assessed by suitable methods. The selection criteria of the fertile group (60 male) were having a child born in the last year and the absence of endocrinopathy, varicocele, and asthenospermia.
Results: Peroxiredoxin activity, Glutathione peroxidase activity, Glutathione peroxidase 4 concentration and total antioxidant status were significantly lower while the concentration of reactive oxygen species and lipid peroxidation were significantly higher in in subfertile men than in healthy men.
Conclusion: Peroxiredoxin activity was inversely proportional with ferroptosis inclination.
Keywords: Ferroptosis, Peroxiredoxin, Glutathione Peroxidase, Total Antioxidant Status, Reactive Oxygen Species

P-7: Evaluation of Sun5 Exons Variations and Sun5 Protein in Patients with Acephalic Spermatozoa Syndrome

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Background: Acephalic spermatozoa syndrome is a rarely tratozoospermia defect. The main anomaly is acephalic and abnormal head-tail junction's spermatozoa. Mutations in SUN5 gene are known as one of the causes of this syndrome. The aim of the present study was to evaluate variations of SUN5 exons in infertile men with this syndrome and assess SUN5 protein in them.
Materials and Methods: DNA was extracted from peripheral blood and after PCR and sequencing the results were analyzed. The protein assessment performed by Immunocytochemistry and Western blotting of semen samples. 10 infertile men with severe acephalic spermatozoa syndrome and 10 men as controls were recruited in this study. It took 2 years to collect these samples.
Results: Sequencing results identified one missense mutation in exon 13 (c.1073G>A [p.Arg358Gln]) in one patient, notably we did not find any mutations in controls. The protein assessment confirmed the genetic outcomes and we observed a complete deletion of SUN5 protein in patient with mutant SUN5.
Conclusion: According to our findings, mutations in SUN5 gene which disrupt the protein expression could be one of the causes of acephalic spermatozoa syndrome. Also, this syndrome could consider as a genetically original syndrome and more accurate studies on familial cases would help to discover some of the other main reasons of this syndrome.
Keywords: Male Infertility, Acephalic Spermatozoa Syndrome, SUN5 Gene

P-8: Association of Heat Shock Protein A2 Expression and Sperm Quality after Supplemental of N-Acetyl-Cysteine in Asthenoteratozoospermia Infertile Men

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Background: In infertile men, it has been demonstrated that heat shock protein A2 (HspA2) is expressed in male reproductive system that causes loss of spermatogenic function. This study was conducted to evaluate the effects of N-acetylcysteine (NAC) with therapeutic dose on expression of heat-shock protein A2 (HSPA2) genes in the sperm and correlation with sperm parameters.
Materials and Methods: Semen samples from 50 asthenoteratozoospermia men with (normal morphology lower than 4% and total motility lower than 40%) were evaluated, who consumed NAC (600 mg/d) orally for three months, after which they were compared with pre-treatment. Sperm parameters analyzed according to World Health Organization (WHO - 2010). The percentage of DNA fragmentation was assayed with TUNEL, and protamine deficiency by Chromomycin A3 (CMA3) test. Sperm HSPA2 expression was determined by using quantitative Real Time PCR (qRT-PCR), and Western blot analysis. Seminal plasma level of stress oxidative factors was measured ELISA kit.
Results: Results revealed that expression levels of HSPA2 was significantly increased in the sperm of NAC-treated relative to pre- treatment ($P<0.05$). A significant improvement in sperm concentration, motility, abnormal sperm morphology, and sperm chromatin integrity after NAC treatment ($P<0.05$). A significant increase in total antioxidant capacity (TAC) and decrease in Malondialdehyde (MDA) level after treatment by NAC ($P<0.05$). Furthermore, the present study showed a significant correlation was found between HSPA2 expression, sperm quality, and stress oxidative factor ($P<0.05$).
Conclusion: NAC may have been increased HSPA2 gene as a protective mechanism against stress oxidative in sperm of asthenoteratozoospermia men.
Keywords: N-Acetyl-Cysteine, HSPA2 Expression, Sperm Chromatin, Asthenoteratozoospermia

P-9: Intracytoplasmic Sperm Injection Outcome Using Ejaculated and Testicular Sperm in Mosaic Klinefelter Patients: A Retrospective Study

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Background: With estimated occurrence of 1 in 7 in the infertile men with non-obstructive azoospermia, Klinefelter syndrome (KS) is the most frequent type of gonosomal aneuploidy in human. Although some of the underlying genetic determinants of the clinical manifestation in KS during the last 60 years unveiled, some characteristics of the mosaic form of it has remained to be elucidated.
Materials and Methods: The study conducted on 145 men

with mosaic KS with the aim to identify any relationship between the spermogram and chromosomal mosaicism as the primary goal, and to evaluate the clinical pregnancy (PR) and live birth (LB) success rates after intracytoplasmic sperm injection (ICSI) using ejaculated and testicular sperm in couples having a partner with mosaic KS, as the secondary outcome.
Results: Of 145 cases, 21 (14.5%) had sperm in semen and the rest (85.5%) classified as azoospermic. In the former group, both of PR and LB per embryo transfer (ET), per cycle and per patient were 22.7% (5/22), 38.5% (5/13), and 45.5% (5/11), respectively. In the latter group, the PR per ET, cycle and patient were 13.6% (3/22), 27.3% (3/11) and 33.3% (3/9), respectively. Of three clinical pregnancies, two were ended with live birth. The mean of age in both groups was 36.7 ± 6 years.
Conclusion: The study provides an update on current knowledge regarding the karyotype-phenotype interrelationships in men with "X-tra" chromosome and highlights the role of inter-individual differences in ICSI outcome.
Keywords: Klinefelter Syndrome, Azoospermia, Mosaic, Intracytoplasmic Sperm Injection, Male Infertility

P-10: Improving The Reproductive System Function of Methylglyoxal-Induced Male Diabetic Rats by Crocin

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Background: Diabetes has recently been a serious problem in the world. Sexual and reproductive disorders are one of the most important secondary complications in patients with diabetes. The present study researches the effects of methylglyoxal (MGO) and crocin on the reproductive system of diabetic male mice.
Materials and Methods: 60 male mice, one-month-old (20-25 g), were chosen for this experimental study and were divided into 6 groups (n=10): sham, MGO, MGO + crocin 15, 30 and 60 mg/kg and also crocin 60 mg/kg alone. Diabetes was induced orally by methylglyoxal (600 mg/KG). During 1 month, Methylglyoxal and crocin was administered. At the 31st day of the study, plasma and tissue samples were separated for experimental assessments.
Results: Blood glucose and insulin levels in the MGO group is higher than in the sham group (P<0.001) and have decreased with treatment (P<0.01). Testis width and volume decreased in the MGO receiving mice. These parameters improved in crocin treated mice (P<0.05). As an antioxidant component, superoxide dismutase level decreased in diabetic mice (P<0.05) and Malondialdehyde enhanced as an oxidant component (P<0.001). Administration of crocin improved these variables (P<0.05, P<0.001 respectively). Luteinizing hormone (LH), testosterone (P<0.001), and sperm count (P<0.05) decreased in the diabetic mice, which crocin treatment recovered them (P<0.01). Vacuoles and apoptosis have been seen in testicular tissue, which crocin improved testicular morphology (P<0.01).
Conclusion: Male reproductive system is affected by MGO induced diabetes. This diabetic model enhances oxidative stress, decrease antioxidant capacity, reduces sex hormones and results in histological problems. Crocin treatment improved these parameters.
Keywords: Crocin, Diabetes, Methylglyoxal, Oxidative Stress, Reproductive System

P-11: Male Infertility and Increased Cancer Risk

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Background: Male infertility is a common disease affecting approximately 30 million men around the world. Epidemiological studies have identified an association between male infertility and increased cancer risk. It has been suggested that various cancer phenotypes may co-occur in men with reproductive disorders. We aimed to identify the differentially expressed genes and the most important pathway associated with cancer and male infertility by Microarray data analysis.
Materials and Methods: The GSE145467 profile was downloaded from the GEO database. GSE145467 submitted by Hodžić A et al. and included 10 testis samples with normal spermatogenesis and 10 testis samples with impaired spermatogenesis. The raw data were subjected to significance analysis with several packages of R statistical software (version 1.2.5033 https://www.r-project.org/). We performed a t test to identify differentially expressed genes. Adjusted P value<0.05 was considered to have a statistically significant difference whereas logFC≥1 was up-regulated genes and logFC≤-1 was down-regulated.
Results: Significance analysis found differentially expressed genes in testis samples with impaired spermatogenesis compared with normal samples. The most important down-regulated genes associated with infertility are genes in the cell cycle regulation pathway (P value=2.49E-05).
Conclusion: Pathway analysis revealed that there might be an undeniable correlation between cell cycle dysregulation and cancers in infertile men.
Keywords: Infertility, Cancer, Cell Cycle

P-12: Effect of Biolozax- H in Sperm Motility in Adult Rat

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Background: Among the etiological factors involved in male infertility was chronic high blood pressure. Drug dose and period of taken of this are important factors. This information can be very useful in the event of an alteration of spermatogenesis function. Biolozax-H, a combination of hydrochlorothiazide and losartan acts as an angiotensin- receptor blocker that treats hypertension and should be further monitored during fertility.

Materials and Methods: Male adult rats (200-250 g) were divided into two groups of eight each. Biolozax-H treated group was administered 20 mg/kg biolozax-H for 90 days. The animals were sacrificed. Immediately after sacrifice, left caudal epididymis was cut and incubated for 3 minutes in pre warmed 37°C normal saline. The sperm solution was placed in a hemocytometer and to evaluate sperm motility, 10 fields were randomly selected and sperms were categorized as different motile or immotile types. The achieved results were analyzed in SPSS software using t test and ANOVA.
Results: Sperm motions were extracted from the testicular epididymis. It was observed that the percentage of fast motility sperm parameter in the experimental groups was decreased significantly. Also the number of immobile sperms in the biolozax-H group decreased significantly compared to the control group ($P<0.05$).
Conclusion: This study showed that the use of biolozax-H for 90 days of treatment can affect the quality of sperm motility. Therefore, further studies on the side effects of this drug on other parameters affecting the process of spermatogenesis are necessary.
Keywords: Sperm Motility, Biolozax-H, Spermatogenesis

P-13: Microfluidic Sorting Selects Sperm as A Novel Method for Clinical Use in Fertilization Failure Patients Undergoing ICSI

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Background: Fertilization failure is an average estimated incidence of 1-3% among reproductive age couples in intracytoplasmic sperm injection (ICSI) cycles. Fertilization failure exists as a frustrating experience. Half of the fertilization failure is due to sperm. Sperm preparation techniques in ICSI procedures is used in order to obtain the best-quality sperm. Current techniques for semen processing in the IVF laboratories, includes centrifuge and incubation steps. The studies show that centrifugation can damage sperm DNA, and sperm DNA damage may lead to a fertilization failure. The aim of this study is to compare the effects of microfluidic sperm sorting from unprocessed semen without centrifugation, as a novel method, and density gradient method on the sperm parameters, DNA fragmentation (DFI) of sperms, fertilization rates and embryo quality in fertilization failure patients undergoing ICSI cycles.
Materials and Methods: Semen samples obtained from fertilization failure patients (n = 12) groups were prepared with density gradient and microfluidic chip methods. The sperm parameters and DNA fragmentation of sperm samples were evaluated before and after preparation. DNA fragmentation of sperm were assessed by SCD method. In addition, fertilization rates and embryos quality were compared between density gradient and microfluidic in the patients with recurrent fertilization failure.
Results: The microfluidic chip method significantly improved sperm motility and morphology compared to density gradient method ($P<0.05$). DFI was decreased in the microfluidic group compared to the density gradient significantly ($P<0.05$). Fertilization rates and embryos quality were significantly increased in the microfluidic group compared to the density gradient ($P<0.05$).
Conclusion: Microfluidic sperm sorting yielded spermatozoa with higher quality, lower DFI and enhanced chances of successful fertilization as well as higher embryos quality compared with conventional sperm preparation methods in fertilization failure patients undergoing ICSI cycles. It is possible that centrifugation steps damage sperm and generates reactive oxygen species (ROS). The advantage of the microfluidic chip may be that centrifugation is not necessary for this method. The result of this investigation has been recommended to use Microfluidic chip method for sperm preparation in fertilization failure couples.
Keywords: Fertilization Failure, Microfluidic Chip, Density Gradient Method, Sperm, DNA Fragmentation

P-14: Can COVID-19 Affect Male Fertility? What May be the Mechanism?

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Background: COVID-19 quickly spread throughout the world. At first, just respiratory damages were in focus. ACE2, the receptor of virus, has high expression in testis. Therefore the virus may attack to testis.
Materials and Methods: We reviewed systematically all the published data, regarding the presence of virus in semen and testicular cells of patients. Also, the evidences and mechanisms behind the testicles damage were investigated.
Results: Although still there are not sufficient studies on the presence of virus in semen and testis, the virus was detected in seminal plasma of some patients (at acute phase, recovered. Also, orchitis was detected in some patient's testes after autopsy. ACE2 has different expression pattern in testicular cells of azoospermic and non-azoospermic patients. Also, "Single-Cell Transcriptomics Analysis" of human testicular cells showed expression of ACE2 and TMPRSS2 in testicular cells. "Pseudo Time Analysis" showed spermatogenesis disruption in COVID-19 patients. "Gene Ontology" (GO) studies confirmed increase of GO, related to reproduction and transmission and decrease of GO, related to sperm production. "Bioinformatics Analysis" claims that sperm damaged in COVID-19. Disruption of Blood-Testis-Barrier happens in acute phase. There is a sex difference susceptibility to infection; men are more susceptible than women. Gonadotropin and testosterone secretion change and Testosterone/ Luteinizing hormone ratio altered. Fever, inflammation, ROS production, cytokine storm, obesity, smoking and drugs can intensify testes damage. Antioxidant therapy may ameliorate the adverse effect of virus on testis.
Conclusion: Male factor fertility should be followed after COVID-19. Also, the risk of sexual transmission should be considered, due to the presence of virus in semen.
Keywords: COVID-19, Testis, Male Infertility, ACE2

P-15: ART3 Polymorphism in Patients with Non-Obstructive Azoospermia

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Background: Infertility is one of the major growing health problems worldwide. Azoospermia (lack of sperm in semen) is one of the main leading causes of male factor infertility among several factors. Azoospermia has two categories: obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Beside several known factors, some genes polymorphism such as ART3 plays role in NOA witch is highly expressing in testis. The aim of the current study was to investigate the association of ART3 polymorphisms with NOA among Iranian males suffering from unknown cause of NOA.
Materials and Methods: After acupuncture from recruited 30 patients with NOA and 88 healthy controls, DNA was extracted from blood samples by saturated salt method. Then, restriction fragment length polymorphism-PCR (RFLP –PCR) was used for evaluation of ART3 gene rs6836703 polymorphism.
Results: This study showed that the rs6836703 of the ART3 gene was statistically correlated with - NOA conditions compared to healthy males (P=0.036).
Conclusion: According to our finding, ART3 polymorphism is associated with NOA conditions.
Keywords: Male Infertility, Non-Obstructive Azoospermia (NOA), ART3 Polymorphism, Restriction Fragment Length Polymorphism (RFLP)

P-16: The Effects of Copper Sulfate on Sperm Quality Parameters, DNA Fragmentation Rate and Testicular Tissue of Adult Wistar Rats

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Background: Copper sulfate is one of the most important environmental pollutants that has the ability to produce free radicals and create oxidative stress. The aim of this study was to investigate the effects of copper sulfate on sperm quality parameters, DNA fragmentation rate and testicular tissue of adult Wistar rats.
Materials and Methods: In this experimental study, 30 adult Wistar rats weighing grams were used. Random adult mice were treated in 3 groups: 1. control, 2. copper sulfate receptor with concentration of 100 mg/kg and 3. copper sulfate receptor with concentration of 200 mg/kg for 56 days. At the end of the treatment period, testicular weight, sperm count and parameters were assessed based on World Health Organization (WHO - 2010) criteria. The quality of sperm chromatin was assessed by acridine uranium nuclear pigments. Malondialdehyde level was measured. Data were analyzed using the One-Way ANOVA statistical method.
Results: The quality of sperm parameters in copper sulfate with a concentration of 200 mg/kg decreased significantly (P<0.05). The testicular weight was significantly reduced at a dose of 200 mg/kg (P<0.05). The diameter of the seminiferous tubules, testosterone levels, spermatogonia count, and sperm DNA fragmentation rate decreased at a dose of 200 mg/kg (P<0.05). The concentration of Malondialdehyde at a dose of 200 mg.
Conclusion: This study shows that a high concentration of copper sulfate causes destructive effects on sperm quality and testicular tissue.
Keywords: Copper Sulfate, Sperm Quality, Testicular Tissue, DNA Fragmentation

P-17: Evaluation of The Effect of N-Acetylcysteine on Human Sperm Parameters and DNA Damage in Frozen-Thawed Sperm Samples of Asthenospermia Men

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Background: The freezing of semen is required not only for fertility, but sperm banking is usually done for Men with Chemotherapy, radiotherapy, surgery and defects ejaculated. The aim of this study was to investigate the effects of N-acetyl-cysteine on human sperm parameters and DNA damage in frozen-thawed sperm samples of asthenozoospermia patients.
Materials and Methods: Samples of 20 patients with asthenozoospermia referred to Qom fertility and Infertility Treatment Center were evaluated in three groups: control, freezing, freezing + N-acetyl-cysteine (1mg/ml). Sperm parameters, viability, and DNA damage were assessed using the World Health Organization (WHO - 2010), Eosin- Nigrosin Staining and SCD kit, respectively in all three groups. Statistical analysis was performed using Repeated measures of ANOVA test and the difference in mean levels of P<0.05 was considered significant.
Results: The freezing process resulted in a decrease in sperm parameters, and the addition of the antioxidant N-acetyl-cysteine improved sperm motility, morphology, and sperm viability. The addition of N-acetyl-cysteine can reduce DNA damage after freezing (P<0.05.).
Conclusion: Our results show that N-acetyl-cysteine can reduce the disruptive effects of the freezing-thawing process in the N-acetyl-cysteine group.
Keywords: Asthenozoospermia, N-Acetyl-Cysteine, Sperm Freezing, DNA Damage

P-18: Protective Effect of Folic Acid on The Deltamethrin-Induced Toxicity on Sperm Quality and Testosterone on Mice

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Background: Deltamethrin (DM) as one of the most widely used agricultural pesticides, is one of the environmental factors that can have destructive effects on the male fertility. According to studies, folic acid (FA) is one of the effective factors in increasing the quality of male fertility.
Materials and Methods: In this experimental study, 25 adult NMRI male mice were divided in to five groups (n=5/each). The control group received only normal saline. Sham received 0.2

ml corn oil. FA group received 0.08 mg/kg, DM group received 0.6 mg/kg and DM+FA group received both of them. After 28 days of treatment, the mice were anesthetized and blood samples were taken from the heart to extract serum. The mice were then operated and the left tail of the epididymis was removed to extract adult sperm. Hormonal analysis was performed by ELISA and CL methods.
Results: The results showed that in DM group sperm count, motility, testosterone and free testosterone significantly decreased (P<0.001). Treatment with FA in the DM-treated mice significantly improved these changes.
Conclusion: With these findings it was concluded that FA can protect against deltamethrin-induced damage and improve epididymal sperm parameters and increase fertility in male mice.
Keywords: Deltamethrin, Folic Acid, Sperm Quality, Testosterone

P-19: Assessment of Sperm Lipid Peroxidation and Intracellular ROS in Type 1 and 2 Diabetes Mellitus Male Mice C57

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Background: Diabetes condition could affect the process of spermatogenesis due to chronic hyperglycemia and increased oxidative stress. In this study, we aimed to evaluate sperm lipid peroxidation and intracellular reactive oxidative stress (ROS) in diabetes mellitus type1 (DM1) and 2 (DM2) male mice C57.
Materials and Methods: Twenty male mice C57 (8 weeks) were divided into 4 groups. Control and sham groups were fed with normal diet, and mice in sham group were induced by a single dose of sodium citrate buffer (0.005mg/kg) as a soluble of streptozotocin (STZ). In addition, DM1 group was induced with 50 mg/kg/day for 5 consecutive days (normal diet), while DM2 group was induced with a single dose of 120mg/kg STZ (60-k cal% high-fat diet). After six weeks, animals were sacrificed, and extracted sperm from the cauda epididymis were used for assessment of the level of sperm lipid peroxidation (Bodipy staining) and intracellular ROS (DCF staining).
Results: The mean percentage of sperm lipid peroxidation was significantly higher in DM1 and DM2 groups compared to control and sham groups (P<0.05). The mean percentage of sperm intracellular ROS was significantly higher in DM1 group than control group (P=0.02). In addition, the mean percentage of sperm lipid peroxidation was higher in DM2 group compared to control group (P=0.000).
Conclusion: Oxidative stress level in type 1 and 2 diabetes mellitus male mice could effect on fertility potential. This result showed that increase of sperm oxidative stress in DM1 and DM2 model mice were high compared to control and sham groups.
Keywords: Type 1 and 2 Diabetes, Oxidative Stress, Infertility

P-20: Assessment of Sperm Parameters in Vitamin D Deficiency Male Mice C57

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Background: Vitamins could be impressed on reproductive system. One of the Vitamins that investigators have focused on fertility is Vitamin D. Therefore, the aim of this study was to induce Vitamin D deficiency in male mice C57 and assess sperm parameters in this condition.
Materials and Methods: Twenty male mice C57 (weight 14 gram- 4 weeks) were divided into control (n=10) and Vitamin D Deficient (VDD) groups (n=10). The control and VDD groups were fed with standard-chow diet, and VDD diet (a diet that have the shortage of vitamin D), respectively. After three months, all of the mice were sacrificed and sperm parameters including sperm motility, sperm concentration, sperm lipid peroxidation (Bodipy staining) and intracellular reactive oxidative stress (ROS) (DCF staining) were done. All of statistical analyses were carried out by IBM SPSS Statistics version 20 and the graphs were designed by Graph Pad prism 8.2. The statistical difference was presented by P<0.05.
Results: In this study, significantly sperm concentration (P=0.000) and sperm motility in progressive (P=0.001) and total motility (P=0.00) in VDD group were lower than control group. In addition, sperm lipid peroxidation and sperm intracellular ROS in Vitamin D deficient group were remarkably higher than control group (P=0.000).
Conclusion: Vitamin D deficiency has deleterious impressions on reproductive system that could lead to disrupt spermatogenesis process and attenuate healthy male fertility.
Keywords: Vitamin D, Sperm Parameters, Infertility

P-21: The long-Term Effect of Oxidative Stress on Sperm Morphology and Lipid Peroxidation in Varicocele-Induced Rats as Time Passes

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Background: Varicocele (VCL) is known as the dilation of the veins of the pampiniform plexus within the spermatic cord. The causative factors of it are multifactorial. It tends to occur more in the left testicle that one of the most potentially increases them is the oxidative stress. An oxidative stressor is such as imbalance temperature which effects on spermatogenesis process deleteriously. Therefore, our aim was the assessment of sperm lipid peroxidation and abnormal morphology, also in more detail in sperm forms mediated by varicocelized rat in long-term (2 months Vs. 4-month varicocele-induced group).
Materials and Methods: We randomly assigned a total of 20 Wistar male rats to 4 groups including VCL-2 [varicocele 2 months (n=5)], VCL-4 [varicocele 4 months (n=5)], control-2 [2 months (n=5)], and control-4 [4 months (n=5)]. Left VCL models were made by partially ligating left kidney veins for the

experimental groups. Rats of control and experimental groups were sacrificed 8 weeks and 16 weeks later respectively. The caudal sperm of epididymis were extracted and sperm lipid peroxidation and abnormal total sperm form and also further details of head, neck and tail of spermatozoa were analyzed. All of the statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS Inc., Version 25.0) and an independent student t-test was used for comparison of variations between two groups. P<0.05 was considered statistically significant.
Results: The mean of sperm lipid peroxidation and total sperm abnormal morphology was significantly higher in VCL-4 month in comparison VCL-2 month (P<0.05), while no observed difference between control groups (P>0.05). Moreover, the mean percentage of sperm abnormal head (P<0.05), sperm abnormal neck (P<0.05) and sperm abnormal tail (P<0.05) were significantly higher in VCL-4 month group in comparing VCL-2 month, though no difference between control groups (P>0.05).
Conclusion: Our results represent that varicocele may produce further oxidative stress in the long-term and has harmful effects on spermatogenesis via probably an increase in heat stress.
Keywords: Varicocele, Sperm Parameters, Sperm Morphology, Reactive Oxidative Stress (ROS),

P-22: Impact of Vitamin E Supplementation on Sperm Parameters after Experimentally Induced Varicocele in Rat

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Background: Varicocele is probably one of the most controversial subjects in the area of male infertility. It is characterized by the dilation and tortuosity of pampiniform plexus (especially in the left testis) and leads to some pathological problems in the testicular tissue. The stasis of venous blood in the dilated pampiniform plexus impairs arterial blood flow and reduces oxygen supply to testis tissue which can lead to testicular hypoxia, hyperthermia, and consequently oxidative stress (OS). OS is known as the main factor in the pathophysiology of varicocele and an increase in reactive oxygen species (ROS) level and decreased antioxidant capacity have been reported in numerous cases. Vitamin E is known as the main antioxidant component of the spermatozoa and a major cell membrane protector against the ROS. This study aimed to create an experimental varicocele model on rats to investigate the impact of vitamin E supplementation on sperm parameters.
Materials and Methods: A total of 40 Wistar rats were randomly divided into four groups: 20 rats just received water and vitamin E as two control groups. And left-side varicocele was induced surgically in 10 rats as the varicocele group. Also in the fourth group, 2 months after varicocele induction rats were gavaged with vitamin E for the next 2 months. 4 months after surgery, animals were euthanized and their genital system dissected and sperm parameters, lipid peroxidation (Bodipy staining), and chromatin integrity (Acridine orange staining) were evaluated.
Results: Our results showed that vitamin E not only did affect positively sperm concentration and motility but also decreased sperm lipid peroxidation and DNA damage in both varicocele induced group and control group.
Conclusion: The present study confirmed the findings of the varicocele effects on lipid peroxidation increase and as a result a significant reduction in sperm motility. Overall, there is evidence in the kinds of literature that defective sperm function is commonly induced by OS, affect sperm motility by lipid peroxidation, DNA integrity by base oxidation. So in individuals with varicocele disorder, antioxidants administration like vitamin E can be a useful approach to scavenge or remove the damage of OS stress created by excessive ROS production.
Keywords: Varicocele, Vitamin E, Sperm, Lipid Peroxidation

P-23: The Effects of Annexin V-MACS Sperm Selection Method on Sperm Parameters, Fertilization and Embryo Development in Male Factor Infertile Couples with High DNA Fragmentation

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Background: Infertile men have deficiency in structure and function of sperm. Sperm selection based on morphology and motility in assisted reproductive technology (ART) techniques is not enough for choosing the best sperm especially in male factor patients. In Annexin V-magnetic activated cell sorting (MACS) technique, apoptotic sperm are separated from non-apoptotic one by negative selection. So, this method can help selecting good quality sperm for Intra cytoplasmic sperm injection.
Materials and Methods: Semen samples from 30 male factor infertile couples (DNA fragmentation index (DFI) >30%) were selected and divided into two group in each patient. control was washed with DGC and experimental one was selected by MACS-DGC. Retrieved eggs in each patient, were divided in 2. Control and experimental group were injected by DGC and MACS respectively. Lots of patients had 2-3 times of implantation failure. Semen parameters and DFI (SCD test) were analyzed before and after processing. After ICSI, the rate of fertilization and embryo development were evaluated. The comparison between results of control and experimental groups was assessed by SPSS analysis.
Results: The results showed that, sperm motility and morphology after MACS method (45%, 1.7%) was significantly higher than DGC method (40%, 1.1%) and before washing (35%, 0.9%). The percent of DFI in MACS group (36%) was significantly decreased compared to DGC (45%) and primitive group (55%). The number of oocytes were injected in DGC group was 93 and in MACS group was 111. Fertilization rate in both groups was almost the same (72.07% in MACS vs 73.11 in DGC). The rate of day 3 embryo with good grade in MACS group (72. 5%) was significantly higher than DGC (51.47%). In addition to, the rate of compaction in MACS (80%) was significantly higher than DGC method (51%) (P<0.05). The pregnancy rate from MACS embryos was 35.4%.
Conclusion: The results indicated, sperm selection by MACS-DGC method can improve sperm motility and morphology and reduce sperm DNA fragmentation although no significant difference was observed in fertilization rate, but the percent of high-quality embryo was significantly higher by this method. In addition, all pregnant had very high DNA fragmentation (> 45%). It seems that, according to the mechanism of MACS method, it can be suggested as a good choice for patients with

high DFI.
Keywords: Annexin V MACS, DNA Fragmentation Index, Male Factor Infertility, Embryo Quality,

P-24: The Effect of Coumarin as An Active Ingradient in Urtica Dioica on Sperm Count and Testosterone in Mice

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Background: Urtica dioica as a medicinal plant has various active ingradients, one of the most important of which is coumarin.
Materials and Methods: In this experimental study, 20 adult NMRI male mice were divided in to four groups (n=5/each). The control group received only normal saline. The experimental groups also received coumarin at doses of 0.25, 0.5, and 0.75 (mg/kg) respectively. Daily injections were administered intraperitoneally for 30 days. The mice were anesthetized and blood samples were taken from the heart to extract serum. The mice were then operated on and the left tail of the epididymis was removed to extract adult sperm. Hormonal analysis was performed by ELISA and CL methods.
Results: The results showed that in a group of mice that received coumarin, sperm count, testosterone and free testosterone significantly decreased (P<0.001).
Conclusion: With these findings it was concluded that coumarin as an active ingradient of Urtica diouca, contrary to expectations, reduces epididymal sperm parameters, testosterone and free testosterone. As a result, it may have a potential reproductive toxicity in adult male NMRI mice. Further studies, are thus needed to determine its mechanism of action upon spermatogenesis.
Keywords: Coumarin, Urtica Dioica, Sperm Count, Sperm Quality, Testosterone

P-25: Protective Effect of The Co-Administration of Testosterone and Sodium Hydrosulfide on Oxidative Stress and Histopathology of Testis in Experimental Model of Varicocele

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Background: Steroidogenesis potent of leydig cells is decreased in varicocele. Hydrogen sulfide (H2S), a novel gaseous molecule, is shown to have protective effects in different organs. The present study was designed to evaluate whether co-administration of testosterone and Sodium Hydrosulfide (NaHS) has protective effects on oxidative stress and pathology of testis in varicocele-induced male rats.
Materials and Methods: Adult male rats were randomly assigned to 5 groups: sham, varicocele, varicocele + testosterone, varicocele + NaHS, varicocele + tetstosterone + NaHS. In the varicocele groups, the left renal vein was partially ligated. In treatment groups, five weeks after the induction of varicocele, testosterone (200µg/kg, subeffective dose) was given subcutaneously for four weeks and NaHS (15µmol/L in drinking water, subeffective dose) were given for four weeks. The right testis tissue samples resected for evaluation of oxidative stress (Malondialdehyde (MDA) and Superoxide dismutase (SOD)) parameters. The left testis tissue also resected and kept in formalin 10% for histopathology studies by hematoxylin and eosin (HandE) staining.
Results: Varicocele caused significant reduction in SOD levels and number of spermatogonia and sertoli cells, and significant increases in MDA levels, compared with the sham group. Administration of testosterone + NaHS significantly increased SOD levels, number of spermatogonia and sertoli cells and significant decreases in MDA levels in varicocele rats compared with varicocele group. But there were no significant changes in these parameters in varicocele + NaHS and varicocele + testosterone group compared with the varicocele group.
Conclusion: This study suggested that long term testosterone and NaHS co-administration could improve oxidative stress and histopathology of testis in varicocele male rats. Therefore, testosterone + NaHS appears to be a useful treatment against varicocele.
Keywords: Varicocele, Testosterone, Hydrogen Sulfide, Spermatogonia, Sertoli

The long-term effect of oxidative stress on sperm morphology and lipid peroxidation in varicocele-induced rats as time passes

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Background: Varicocele(VCL) is known as the dilation of the veins of the pampiniform plexus within the spermatic cord. The causative factors of it are multifactorial. It tends to occur more in the left testicle that one of the most potentially them increased oxidative stress is. An oxidative stressor such as imbalance temperature which effects on spermatogenesis process deleteriously. Therefore, our aim was the assessment of sperm lipid peroxidation and abnormal morphology, also in more detail in sperm forms mediated by varicocelized rat in the long-term(2 months Vs. 4-month varicocele-induced group).
Materials and Methods: We randomly assigned a total of 20 Wistar male rats to 4 groups including VCL(varicocele 2months (n=5), varicocele 4month s(n=5)), control (control 2months (n=5), control 4month s(n=5)). Left VCL models were made by partially ligating left kidney veins for the experimental groups. Rats in control and experimental groups for 8 weeks and 16 weeks later were killed, respectively. The caudal sperm of epididymis were extracted and sperm lipid peroxidation and abnormal total sperm form and also further detail in head, neck and tail of spermatozoa were analyzed. All of the statistical analyses were carried out using the Statistical Program for Social Sciences (SPSS Inc., Version 25.0) and an independent student t-test was used for comparison of variations between two groups. P < 0.05 was considered statistically significant.
Results: The mean of sperm lipid peroxidation and total sperm abnormal morphology was significantly higher in VCL-4 month

in comparison VCL-2 month ($p<0.05$), while no observed difference between control groups ($p>0.05$). Moreover, the mean percentage of sperm abnormal head ($p<0.05$), sperm abnormal neck ($p<0.05$) and sperm abnormal tail ($p<0.05$) were significantly higher in VCL-4 month group in comparing VCL-2 month, though no difference between control groups ($p>0.05$).
Conclusion: Our results represent that varicocele may produce further oxidative stress in the long-term and has harmful effects on spermatogenesis via probably an increase in heat stress
Keywords: Varicocele, Sperm parameters, Sperm morphology, Sperm ROS,

Animal Biotechnology

P-26: Prostaglandin Estradiol and Oocyte *In Vitro* Maturation in Goat

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Background: Prostaglandins are a group of signaling molecules that mediate many important reproductive processes, including expansion of cumulus cells, expression of proteases associate with follicle rupture, implantation, and maintenance of luteal function and establishment of pregnancy. This essential derivative of arachidonic acid. Once produced, Prostaglandin E2 (PGE2) acts by binding to specific G protein-coupled receptors. The role of PGE2 during early embryogenesis has been studied in several species, including humans, rhesus monkeys, mice, and various domestic animals.
Materials and Methods: In this study, we measured the effect of PGE2 (0.1nM, 1nM and 10 nM) on goat oocyte in vitro maturation. At first, goat cumulus oocyte complex (COCs) were aspirated from slaughterhouse ovary, washed and exposed for 24 hours to different concentration of PGE2. TCM and conventional medium were considered as control groups. We assessed the effect of PGE2 during *in vitro* maturation (IVM) on goat oocyte nucleus with Hoechst staining, embryonic development and blastocyst morphological quality with differential staining.
Results: All data indicated the maturation rate of oocytes were not significantly difference among concentration of PGE2 incredibly less than conventional group. The cleavage rate assessment was similar in the control and PGE2-treated groups. Moreover, we found no difference in the proportion of oocytes reaching the blastocyst stage on day 7 in the TCM control compared to the PGE2-treated groups but different with conventional group. The analysis of the cell number of blastocyst quality showed that there were not significantly differences in the PGE2-treated groups compared to the TCM control.
Conclusion: This study indicated that PGE2 solely might not control the processes which occurs during oocyte maturation in the goat. PGE2 may be better effect on cumulus expansion and other process of oocyte development in combination of Natriuretic peptide or/and Amphiregulin.
Keywords: Goat, Oocyte, Maturation, Prostaglandin

P-27: Differential Changes of Reproductive System Properties in F1 Male Pups following in Utero and Breastfeeding Exposures to Nicotine and Ethanol, Alone or Concurrently

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Background: Most smokers use ethanol, simultaneously. The aim of this study was to evaluate the effect of in utero and breastfeeding co-exposure to these substances on reproductive system of male offspring.
Materials and Methods: Pregnant and lactating NMRI mice were randomly divided into 4 groups: toxins groups; received nicotine (Nic;1 mg/kg, i.p) and ethanol (received ethanol 3 gr/kg, i.p.), alone and concurrently and vehicle group that received normal saline, i.p. Treatments started from pregnancy day1 until weaning. The testicular and sperm parameters as well as oxidative stress marker; malondealdehyde (MDA) level were investigated in male offspring at postnatal day 90.
Results: We observed a decrease of testicular Johnsen's score, sperm motility, count, viability, DNA integrity and an increase of serum MDA level in toxin-exposed groups. Interestingly, concurrent-exposed pups showed slighter effects when compared to exposure to any of these toxins, alone.
Conclusion: Collectively, our data exhibited that maternal life style such as smoking and drinking during pregnancy and lactation periods could negatively affect male offspring fertility potential by decreasing the testicular and sperm parameters. The stress oxidative may be one of the reasons, partly. However, co-use of these substances had an alleviating effect with unknown mechanisms.
Keywords: Nicotine, Ethanol, In Utero Development and Lactation Periods, Reproductive System, Male Offspring

P-28: Investigation Histological and Biochemical Evaluation of Pups and Liver of Ethanol Treated Pregnancy Mothers in Rat

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Background: Excessive alcohol consumption is one of the most effective factors in causing inflammation of the liver (ALD). Continuous alcohol consumption during pregnancy causes serious damage to the developing fetus, the most important consequences of which can be changes in the developing brain and neurobehavioral defects throughout life, as well as leading to a wide range of teratogenic effects. Drinking alcohol, even in moderation, is associated with an increased risk of miscarriage, especially in the first trimester of pregnancy and infertility in women. Exposure to alcohol at a stage equivalent to the first trimester of pregnancy in humans caused fetal alcohol syndrome (FAS) and was associated with loss of muscle movement during the second and third trimesters. The third trimester has also been shown to have serious effects on the fetus, as it is associated with a significant increase in essential nutrients in the brain and retina. Frequent consumption of alcohol causes liver dysfunction. Alcohol-induced liver disease is one of the most common causes of liver cancer and cancer mortality, and is also a major cause of leukemia. Reactive oxygen species (ROS) has been reported to affect the oxidation of proteins and fats. Oxidation damages DNA, inactivating the enzyme and destroying various antioxidant enzymes. Ethanol consumption chronically

regulates nitric oxide (NO) levels and the expression level of protein cyclooxygenase (COX) in maternal and neonatal liver tissue. Recent studies by Natabaj have shown that ethanol consumption increases the phosphorylation of JNK, ERK and P38 in the liver of mother and offspring of rat.
Materials and Methods: Pregnant rats were randomly divided into 3 experimental groups: the control group receiving distilled water and the oily ethanol group receiving ethanol with oil by gavage up to 28 days after delivery. Mice in the ethanol group received ethanol (4 g / kg) as a solution in distilled water (40% v / v) as oral gavage from day 0 of gestation until 28 days after delivery. On the 18th day before birth and 28 days after birth, a number of rats were sacrificed for histopathological examination of the liver of the mother and the fetus, and the fetus was examined for weight and appearance. Liver tissue samples were placed at -70°C for biochemical tests. And some other animals under deep anesthesia, then perfusion operation was performed and then the animal's liver was removed. For better fixation, we first changed the liver tissue in 10% paraformaldehyde solution every 12 hours and then histopathological examination was performed.
Results: Evaluation of oxidative enzymes showed a significant increase in MDA in the ethanol group compared to the control group ($P<0.5$). Also, GPX level decreased significantly compared to control ($P<0.05$). In histopathology, the number of hepatocytes in the ethanol group increased significantly ($P<0.5$) and caused hepatocyte necrosis and severe weight loss and fetal defect in the ethanol group.
Conclusion: According to the results, ethanol caused severe weight loss and defects before and after birth in the pups as well as damage to hepatocytes, Kupffer cells and maternal liver lobules. The number of Kupffer cells decreased.
Keywords: Pups, Liver, Pregnancy, Rat, Ethanol

The effect of rapamycin on autophagy status in SCNT embryos in goat species

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Background: Although somatic cell nuclear transfer (SCNT) is a promising technology, but its application is limited because of its low efficiency. Autophagy is involved in embryonic development and is necessary to destroy maternal mRNA and eliminate the accumulation of unnecessary proteins and organelles. Limited studies have shown the role of autophagy during preimplantation development in fertilized embryos, but no studies have investigated its role and status in SCNT embryos. Therefore, in this study the comparison and evaluation of autophagy status was performed between IVF and SCNT embryos in goat species.
Materials and methods: Reconstructed oocytes from the SCNT process were collected 3, 6 and 9 hours after activation and then the autophagy status was evaluated by immunofluorescence staining of LC3B protein in goat species. According to studies in the IVF process, sperm penetration occurs 8 hours after insemination. In order to compare the SCNT process with IVF, IVF zygotes were collected at (8 + 3) 11, (8 + 6) 14, (8 + 9) 17 hours post insemination, fixed and immunostained similar to SCNT embryos. In addition, to evaluate the effect of rapamycin on autophagy status in SCNT embryos, the restructured oocytes were incubated in rapamycin (1, 10, 100 nM) for 6 hours and similar to IVF and SCNT embryos they were also immunolabelled with LC3B antibody.
Results: During 6 hours after oocyte activation in SCNT embryos, no induction of autophagy was observed compared to IVF embryos, but treating the reconstructed oocytes with 10 and 100 nM rapamycin increased the LC3B protein expression (as an autophagy marker) in SCNT-derived embryos.
Conclusion: The results of our study show that autophagy is absent in SCNT derived embryos during the first 6 hours after artificial activation compared with IVF derived embryos. The first signs of autophagy was observed in SCNT embryos around 9 hours after activation. In addition treatment of reconstructed oocytes in SCNT group with 10 and 100 nM rapamycin for 6 hours after activation induced the autophagy. These results may be a promising approach for improving SCNT efficiency through activation of autophagy with rapamycin.
Keywords: SCNT, autophagy, rapamycin, LC3B

Evaluation of IWR1 effect, an inhibitor of WNT signaling pathway, on pre-implantation development of IVF and SCNT derived embryos in goat species

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Background: Despite many advances in improving the in vitro fertilization (IVF) technique, its efficiency in terms of post-implantation development is lower compared to natural pregnancy in different species. There are three main messenger pathways involved in embryonic development: WNT, FGF and TGF-β. Among these pathways, the WNT pathway plays an important role in developmental processes. There is limited information about the role of WNT pathway on development of fertilized embryos. Previous studies have shown that activation of WNT pathway during post compaction stages can reduce the pre-implantation development. Regard to this, in this study we assessed the effect of WNT inhibition using a small molecule (IWR1) during post compaction stages on pre-implantation development of IVF embryos in goat species.
Materials and Methods: In order to evaluate the inhibition of WNT pathway on blastocyst rate of IVF embryos, 3 concentrations of IWR1 (1.25, 2.5, 5 µM) were added to culture medium 4 days after fertilization. The blastocyst rate was assessed on day 7 of in vitro development. In addition, total cell number (TCN), inner cell mass number (ICM) and trophectoderm number (TE) was evaluated through differential staining in derived blastocyst from various treated groups.
Results: Our results showed that treatment of IVF derived embryos with 1.25 and 5 µM IWR1 significantly increased the blastocyst rate (32.25±3.58 % and 33.69±5.64 %, respectively) compared to the control group (20.38±2.68 %). The number of ICM, TE and TCN did not change following the treatment with IWR1 in blastocyst embryos.
Conclusion: The results of our study showed that inhibition of WNT pathway with an efficient small molecule, IWR1, can improve pre-implantation embryonic development in terms of blastocyst rate.
Keywords: WNT signaling pathway, SCNT, IWR1, Inhibitor

Embryology

Follicles Activity Reconstruction Using Decellularized Matrix of Intact Ovary Seeded by Ovarian Stromal Cells of Mouse Premature Ovarian Failure (POF) Model Following Auto Transplantation

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Background: Ovarian tissue engineering for follicular activity reconstruction, holds great possibility for female infertility treatment in premature ovarian failure (POF) due to chemotherapy. In this study, we determined whether human ovarian scaffolds could reconstruct the ovarian structure.
Materials and Methods: For the first step, female NMRI mice aged 6-8 weeks were subjected to making POF model, by 5 experimental and control groups. Experimental groups were selected based on different amounts of Cyclophosphamide (C) and Busulfan (B) chemotherapy drugs which were intraperitoneal injected for two weeks (group 1: 75 mg/kg C/14 days, group 2: 100 mg/kg C/14 days, group 3: 100 mg/kg B in 1st day+20 mg/kg B/14 days, group 4: 100 mg/kg C in 1st day+50 mg/kg B/14 days, group 5: 75 mg/kg C and 30 mg/kg B/14 days). The best model of POF, was determined by hormonal assay (FSH and Estradiol (E2)), and expression of germline markers (Oct4, Dazl). In the second step, for creating an artificial ovary, the isolated ovarian cells from POF mouse model were seeded in human decellularized ovarian scaffolds, divided in 4 groups and transplanted in mouse for one and two months as follows: Cont.1 and Cont.2: human ovarian scaffold seeded with intact mouse isolated ovarian cells (OCs) and transplanted in mouse for 1 and 2 months; POF.1 and POF.2: human ovarian scaffold seeded with POF model mouse isolated OCs and transplanted in mouse for 1 and 2 months. Histological and hormonal evaluations (FSH, E2) were assessed.
Results: Due to decrease in estradiol and increase in FSH levels in group 2, the best POF model was confirmed. Also, genes expression of Oct-4 and Dazl showed an increase ($p<0.05$) in group 2 compared to the control one. The histological assessment of the graft after one month of transplantation showed formation of follicular like structures while these structures had grown more and came closer to maturity after two months of transplantation. The results of hormone evaluation determined that there is no significant increase in FSH hormone.
Conclusion: Altogether, our findings produce verification for the use of human ovarian scaffolds as an appropriate platform for maintenance and proliferation of isolated and seeded ovarian cells.
Keywords: Premature Ovarian Failure, Chemotherapy, Tissue Engineering

In Vitro Spermatogenesis of Neonate Mouse Spermatogonial Stem Cells on Human Placenta Decellularized Matrix

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Background: Male infertility affects 7 % of the male population and 10 % of infertile men are azoospermic. Extracellular matrix (ECM) of tissues contains various ranges of growth factors, proteins, proteoglycans, hyaluronic acid and others. Decellularization of tissue and production of tissue engineering scaffolds from ECM components is one of the most reliable strategies in fabrication of scaffolds. So proliferation of spermatogonial stem cells on human placenta decellularized matrix as a scaffold was evaluated.
Materials and Methods: Human placenta was obtained from mothers undergoing cesarean after obtaining informed consent. The tissues were treated with Triton X-100 and sodium dodecyl sulphate (SDS) for 30 minutes. Spermatogonial stem cells were isolated from neonatal mice after enzymatic digestion and flow cytometry and RT-PCR were used to confirm the identity of spermatogonia cells for Plzf, Id4, Gfrα1 and Gapdh genes. Proliferation of spermatogonia cells on the scaffold were investigated using quantitative Real-Time PCR (qPCR) for pre-meiotic (ID4 and Gfrα1) and meiotic (Sycp3) genes during culture process.
Results: Spermatogonial stem cells adhesion, proliferation and colony formation were confirmed by Hematoxylin and Eosin, scanning electron microscope (SEM) and transmission electron microscope (TEM) tests. The proliferation of Id4 gene expression decreased after 14 days of culture in the three dimensional (3D) group. Gfrα1 gene expression increased significantly during the amplification stage and was higher in 3D group than two dimensional (2D) group ($p\leq0.05$). Sycp3 gene expression also increased significantly in 3D group more than the 2D culture group ($p\leq0.05$). Acrosin and Prm1 gene expression also increased significantly after 5 weeks and was higher in the 3D group than the 2D group ($p\leq0.05$). Flow cytometry results showed a significant increase in Gfrα1 factor after 14 days of proliferation in the 3D group and also a significant increase in Gfrα1 compared to the 2D group ($p\leq0.05$).
Conclusion: The decellularized placenta is suggested as a promising bio-scaffold tissue engineering applications that could improve attachment of SSCs.
Keywords: Decellularized Scaffold, Extracellular Matrix, Placenta, Spermatogenesis,

Development of a Composite Alginate-Gelatin- Testicular Extracellular Matrix Hydrogel as A Potential Bioink for 3D Printing of Artificial Testis

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Background: Spermatogonial stem cell (SSC) loss due to cancer treatment, developmental disorder or genetic abnormality may cause permanent infertility. Tissue and cell culture, three dimensional (3D) scaffolds and adding of supplements recently have created a new perspective for the differentiation of stem cells in vitro. Therefore, the purpose of this study was to use sheep testicular extracellular matrix (T-ECM) as a biological material for culture of mouse SSCs in vitro.
Materials and Methods: The extracted T-ECM (with different percentages of 0, 1.5, 3, 5%) was used to print the hydrogel scaffold with alginate-gelatin. After cross-linking using aqueous CaCl2 + glutaraldehyde solution, mechanical tests were performed to evaluate the compressive strength and degradability and swelling tests to evaluate the structural and biological properties of the scaffolds. The surface morphology of the scaffold was examined using scanning electron microscope (SEM). Non-toxicity and scaffolds cell adhesion for SSCs was studied using MTT assay and SEM.
Results: Results of our study showed that increasing the concentration of ECM in printed scaffolds could increase the hydrophilicity of the scaffold and subsequently enhance the swelling properties that in the printed scaffolds with 5% ECM were acceptable. Also, results of SEM and MTT assay demonstrated that the printed scaffolds with 5% ECM had proper surface properties, cell adhesion, and good biocompatibility than other groups.
Conclusion: In general, our study suggested that T-ECM can be used as a bio-ink for design a functional bioartificial testis in vitro and will improve process of spermatogenesis that would offer new fertility restoration options.
Keywords: Spermatogonial Stem Cell, 3D Printing, Artificial Testis, Testicular Extracellular Matrix

Reversal of The Detrimental Effects of Diabetes on The Reproductive and Pregnancy Complications Using Quercetin Treatments in STZ-Induced Diabetic Mice

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Background: Diabetic women have different reproductive problems and impaired fertility. In this study, we investigated the protective effects of quercetin treatment on the uterine receptivity markers, blastocyst implantation rate, development of the preimplantation embryo, and folliculogenesis.
Materials and Methods: Streptozotocin-induced diabetic mice were treated with 30 mg/kg/day quercetin for four weeks. The Blood was collected on day 4 of pregnancy to analyze the serum sex-steroid levels. Blastocysts were collected by flushing dissected uteri and then uterus was harvested at day four of pregnancy for investigation of protein and mRNA expression changes. Furthermore, the right ovary was harvested to stereological analysis.
Results: Serum estradiol level reduced in diabetic mice but, treatment with quercetin significantly increased serum estradiol level. In diabetic mice with quercetin treatment, morphological distribution was shifted considerably to the well-developed stages. Treatment with quercetin was improved expression of some important molecules in the development and implantation. Also, the level of apoptosis was reduced in the blastocyst and uterus of diabetic mice with quercetin treatment. Besides, our results indicated that the administration of quercetin in diabetic mice increased the volume of the ovary and growing follicles as well as the number of growing follicles and corpus luteum.
Conclusion: We propose that the administration of quercetin before conception probably can alleviate reproductive problems in diabetic women likely via its estrogenic and antihyperglycemic effects. Our studies propose a novel and safe treatment of reproductive disorders in women suffering from diabetes.
Keywords: Diabetic Pregnancy, Reproductive Complication, Phytoestrogen, Quercetin

Co-Culture of Human Cryopreserved Fragmented Ovarian Tissue with Theca Progenitor Cells Derived from Theca Stem Cells

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Background: While significant advances have been made on the in vitro development of human preantral follicles, this approach remains challenging and there is still a great potential for its improvement. Based on such need, this study aims to assess the impact of a feeder layer of human theca progenitor cells (hTPCs) on the development of human primordial follicles.
Materials and Methods: To this end, frozen-thawed ovarian fragments were first activated with 15 mM vanadate-derivative dipotassium bisperoxo (5-hydroxy-pyridine-2-carboxylic) oxovanadate (V)) and 100 µg/ml kit ligand for 24 hours and then cultured with or without hTPCs. After 6 days, follicles were counted and classified, hormone levels in the spent medium were measured and the expression of genes involved in apoptosis and folliculogenesis was assessed.
Results: Histological analysis revealed a significant follicle growth in both in vitro culture groups. However, the number of growing follicles was significantly higher in the co-culture group ($p<0.05$), which also exhibited a few antral follicles. In addition, BMP-7, AMH, GDF9 and ZP1,2,3 gene expressions were significantly increased in the co-culture. On the other hand, P53, CASP-3, and BAX gene expressions significantly increased in the mono culture in contrast to BCL2. Co-culturing with hTPCs also dramatically enhanced the concentration of estradiol, progesterone, testosterone and androstenedione ($p<0.05$).
Conclusion: In conclusion, hTPCs significantly improved the development of human primordial follicles, increasing hormone production, promoting cell proliferation and reducing apoptosis.
Keywords: Human Primordial Follicles, Co-Culture System,

Human Theca Progenitor Cells, Human Ovarian Cryopreservation, Ovarian Cortical Fragments

The Antioxidant Effects of Astaxanthin on Oxidative Stress, Apoptosis, and AKT Signaling Pathway in Granulosa Cells of BALB C Mouse Model of Polycystic Ovary Syndrome

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Background: Increasing inflammatory factors and reactive oxygen species (ROS), involved in the pathogenesis of the polycystic ovary syndrome (PCOS), plays a key role in the onset of apoptosis in follicles and granulosa cells (GCs). However, the AKT signaling pathway mediates cell survival and thus has a protective role against apoptosis. Thus, we aimed to investigate the antioxidant effects of Astaxanthin (AST) on GCs using a PCOS mouse model.
Materials and Methods: In this study, 48 prepubertal female mice of BALB C aged 25-30 days and weighing 12-14 g were studied. The PCOS model was created by subcutaneous injection of the dehydroepiandrosterone (DHEA) hormone in 8 mice of BALB C for 20 consecutive days. Following AST dosimetry, the effective dose for the treatment group (0.1 mg/kg) was administered. The GCs were then isolated and purified. Apoptosis and the amount of ROS were evaluated via flow cytometry. The activity of AKT protein was measured by western blot, and the viability of GCs was investigated using spectrophotometry. Tissue sections of ovaries were prepared, stained with hematoxylin and eosin (H&E), and the morphology of the sections was examined. Statistical analysis was performed by SPSS v22.0 software using one-way ANOVA.
Results: AST administration leads to a significant reduction in oxidative stress ($p<0.01$) and, consequently, a significant decrease in the rate of apoptosis ($p<0.01$). The expression of AKT in the AST group revealed a significant increase ($p<0.05$) compared with the control and PCOS groups. Ovulation was confirmed in the AST group.
Conclusion: Our results revealed that the administration of AST could reduce the oxidative stress factor H2O2 and apoptosis, while it increased the level of AKT expression. Ovulation was also observed in the ovaries. Further studies are warranted to prove the efficacy of AST and to introduce it as a complementary therapeutic agent in PCOS.
Keywords: Polycystic Ovary Syndrome, Astaxanthin, Oxidative Stress, Apoptosis, AKT Protein

An Especial Subfraction of Ceratonia Silique L (Carob) Extract Induces Spermatogenesis in An Infertile Mice Model as Well as The Total Extract

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Background: Nowadays, scientists try to improve the adverse effects of male infertility treatment through the use of herbal nutraceuticals. Carob extract is being traditionally used for male infertility treatments. However, there is no scientific evidence for effective components in Carob to be used in male infertility treatments. Herein, we appraised the various "Carob fractions" in the infertile mice model.
Materials and Methods: 28 adult male mice were divided into seven groups: intact, vehicle, positive control (600 mg/kgbw of total extract), and four experimental groups that were administrated by fraction 1 to 4 at 200 mg/kgbw for 35 days by oral gavage. Sperm parameters, testicular histopathology, DNA content, and gene expression analysis for spermatogonial stem cells (SSCs) differentiation were investigated after 35 days.
Results: Our data demonstrated that only one fraction out of five obtained from Carob extract significantly increased sperm count, motility, and also spermatogenesis in histological analysis. Moreover, this fraction significantly reduced DNA fragmentation in sperms. Gene expression analysis showed a significant increase in the expression levels of genes involved in spermatogenesis differentiation, including Dazl, Ngn3, and Stra8, and a notable decrease in C-kit.
Conclusion: We demonstrated one specific fraction obtained from Carob as a natural compound that has the ability to induce spermatogenesis in the infertile model. However, more investigation needs to demystify its function before applying it in the clinic for non-obstructive azoospermia men.
Keywords: Infertility, Spermatogenesis, Fraction, Carob Extract

The Combination of Basic Fibroblast Growth Factor and Kit Ligand Promotes the Proliferation, Activity and Steroidogenesis of Granulosa Cells During Human Ovarian Cortical Culture

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Background: Different factors, such as basic fibroblast growth factor (bFGF) and kit ligand (KL), are used in ovarian cortical culture to promote primordial follicles activation. In the present study, the effects of bFGF and KL combination were evaluated on human follicular activation and growth during in situ cortical culture.
Materials and Methods: Slow frozen-thawed human ovarian cortical tissues (n=6) were cultured in 4 different groups: 1) control (base medium (BM)), 2) KL (BM + 100ng/ml KL), 3)

bFGF (BM + 100ng/ml bFGF) and 4) bFGF+KL (BM + 100ng/ml KL + 100ng/ml bFGF) for a week. Then, the proportion of morphologically normal and degenerated follicles at different developmental stages, secreted hormonal levels and specific gene expressions were compared.
Results: Although the proportion of growing follicles was higher than primordial counterpart in all cultured groups, no significant difference was observed between cultured groups. In all cultured groups, anti-Müllerian hormone (AMH), progesterone and estradiol hormones level were increased after 7 days of culture, however, this increase was only significant for estradiol in the bFGF+KL group. The expression of Ki67 gene indicated an increase in ovarian cells proliferation in the three experimental groups compared to the control group, however this increment was only significant for the bFGF+KL group.
Conclusion: It can be concluded that KL and bFGF factors alone had no significantly beneficial effect on follicular growth in situ, while when their combination was used, a positive effect on steroidogenesis of granulosa cells without significantly increasing the number of growing follicles was observed.
Keywords: Human Ovarian Tissue, Cryopreservation, Kit Ligand, Basic Fibroblast Growth Factor, Primordial Follicle

The Effect of Myo-Inositol on ATP Content, Intracytoplasmic Oxidative Stress (ROS) and Reduced Glutathione (GSH) Levels in The MII Oocyte of DHEA-Induced PCOS Mice

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Background: Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism, decreased ATP content, increased and decreased levels of reactive oxidative species (ROS) and glutathione (GSH), respectively, and ultimately low quality oocytes. Inofolic contains 200 µgr folic acid and 2gr of myo-inositol. The administration of myo-inositol was associated with a decreased of serum testosterone, increase insulin sensitivity and improves the oocytes' quality.
Materials and Methods: The present study was performed in DHEA-induced mice. Female NMRI mice were treated with a vehicle control (Sesame Oil 0.05 ml and saline) or DHEA (6 mg /100 g body weight) or DHEA plus inofolic (37 mg /100g body weight) for 20 consecutive days. After 20 days' superovulation was performed, Mature oocysts (MII) were retrieved from isolated ovaries. For ROS or reduced GSH staining, denuded MII oocytes were incubated in PBS–PVA containing H2DCF-DA and Cell Tracker Blue fluorochromes, respectively. After incubation, fluorescence intensity was measured using invert fluorescence microscopy. ATP assay was also performed by a colorimetric-based commercial mouse adenosine triphosphate ELISA kit on denuded MII oocytes.
Results: ATP content and GSH level were significantly lower and ROS level was higher in DHEA-treated oocytes compared with vehicle-treated. In DHEA+ myo-inositol group, ROS was significantly reduced in contrast to the DHEA group and, on the contrary, GLUT and ATP content had risen.
Conclusion: It seems that myo-inositol improves intracellular Ca2+ signaling and mitochondrial function which ultimately reduces oxidative stress level, increases ATP production and mitochondrial antioxidant capacity in the oocytes of DHEA-treated mice.
Keywords: Myo-Inositol, Reactive Oxidative Stress, Glutathione (GSH), ATP Content, Polycystic Ovary Syndrome

Investigation of signaling pathways understanding Carob function for inducing spermatogenesis in an In Vitro platform

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Background: Impairment in the spermatogenesis process is the main cause of male infertility. Recently, scientists tried to improve the efficiency of male fertility treatment through the use of herbal nutraceuticals extracts. Carob is being traditionally used for male infertility treatments. However, there is no scientific evidence for the principal mechanism effect of Carob on spermatogenesis-related signaling pathways. Herein we evaluate 3 main spermatogenesis-related signaling pathways in mouse testicular cells-enrich for spermatogonial stem cells (SSCs) following treatment with Carob whole extract
Materials and Methods: To evaluate the spermatogenesis-related TGF-β, BMP4, GDNF (MEK related) signaling pathways following treatment with Carob whole extract, after finding non-toxically Carob concentration for testicular cell culture by PI staining (2 mg/ml), isolated cells were treated by the medium containing Carob extract and one of the following small molecules: SB431542, LDN193189 and PD0325901 respectively. Cells were collected for gene expression analysis after 9 days of treatment.
Results: Our primary results suggested that by inhibiting the BMP4 signaling pathway using LDN193189 at the presence of Carob, all of the examined genes (Plzf, Gfr-α1, Bcl-6b, Dazl, Stra8) were significantly decreased compared to Carob treat. Gene expression profiles had different patterns on inhibition of other signaling pathways.
Conclusion: It seems that the BMP4 signaling pathway is the master effector upon Carob function. Activation of this signaling pathway, directly and indirectly, effects on differentiation and self-renewal of SSCs to promote spermatogenesis. However, the carob contains a set of effective compounds that promote spermatogenesis by the effect on most spermatogenesis related signaling pathways.
Keywords: Male Infertility, Spermatogenesis, Carob Extract, Small Molecule, Signaling Pathways

Membrane Lipid Replacement (MLR) with Nano-Micelles in Human Sperm: A New Approach of Sperm Protection During Cryopreservation

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Background: Membrane Lipid Replacement with lipid nanomicelles (NMs) in sperm can be as a new strategy for increasing sperm cryosurvival. This study investigated the effects of NMs made from glycerophospholipid mixtures (GPL) and cholesterol-loaded cyclodextrin (CLC) on frozen-thawed human sperm function.
Materials and Methods: We examined the size and morphology of NMs by dynamic light scattering (DLS) and scanning electron microscope (SEM), respectively. Semen samples were collected from normozoospermic men (n=30). After sperm processing, each sample was divided into ten aliquots according to the fresh, frozen without treatment and those exposed to GPL (0.1 and 1 %) and CLC (1 and 2 mg/ml) at the following groups: GPL-0.1, GPL-1, CLC-1, CLC-2, GPL-0.1/CLC-1, GPL-0.1/CLC-2, GPL-1/CLC-1 and GPL-1/CLC-2 for 1 hour at 37˚C (5 % CO2) before cryopreservation. The optimum kind and concentration of NMs were determined by evaluation of motility parameters using computer assisted semen analysis (CASA) and viability by eosin-nigrosin stain. Moreover, sperm parameters such as apoptosis (Annexin/PI), mitochondrial activity (JC1), acrosome integrity (PSA), and DNA fragmentation (SCSA) were assessed to detect the effect of the optimum group on sperm function.
Results: Exposure of sperm to GPL-0.1/CLC-1 and GPL-0.1 significantly increased total, progressive motility, average path velocity (VAP), straight linear velocity (VSL), and curvilinear velocity (VCL), and the percentage of viability was significantly higher in the CLC-1, GPL-0.1, and GPL-0.1/CLC-1 groups compared to the frozen control group ($p<0.05$). Furthermore, a significantly higher percentage of sperm mitochondria activity and acrosome integrity, and the lowest rate of apoptosis were observed in GPL-0.1/CLC-1 group in comparison to the frozen control group. DNA fragmentation of thawed sperm were not affected by NMs ($p>0.05$).
Conclusion: Our findings indicated that membrane lipid replacement with NMs (GPL-0.1/CLC-1) could substitute damaged lipids in membrane and protect sperm cells against cryoinjury.
Keywords: Cryopreservation, Human Sperm, Nano-Micelle, Glycerophospholipid, Cholesterol-Loaded-Cyclodextrin

Effects of Leukemia Inhibitory Factor (LIF) on Human Embryo Development and Attachment in An Endometrial Three Dimensional System Culture

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Background: Leukemia inhibitory factor (LIF) is one of the key extrinsic factors that could be largely used for embryo culture. LIF is expressed at its highest level in endometrial cells at mid luteal phase when the embryo is at the blastocyst stage. In this study, the development of the 8 cell human Embryos into blastocyst stage and their attachment to the designed three dimensional (3D) structure were assessed at the presence and absence of LIF.
Materials and Methods: An in vitro 3D endometrial model was constructed by a mixture of endometrial stromal cells with collagen and fibrin gel that covered with two layers of matrigel and isolated epithelial cells, respectively. Eight cell human embryos (24 embryos in each group) were cultured on top of the 3D construct 4 days after starting culture, with 1000IU LIF (experimental group) or without LIF (control group). Blastocyst growth, cell count and blastocyst attachment to the 3D structure were evaluated. Also expression level of Bax, Bcl2, Klf4, microRNA 145 and 372 were analyzed in obtained blastocysts by quantitative Real-Time PCR 3 days after starting embryo culture.
Results: There was no significant difference in the embryo development between the LIF treated group and the control group, whereas attachment rate between this groups was different as 14 out of 24 human embryos exposed to LIF (73.7%) and 10 out of 24 embryos in the LIF free group (55.5%) attached to the apical surface of endometrial constructs. Total cell number of blastocysts in the LIF group was significantly higher than the LIF free group. LIF up-regulated expression of Klf2 and down-regulated microRNA 145 in expanded blastocysts, but it had no effect on the Bax/Bcl2 ratio and microRNA 372 expression in obtained blastocysts.
Conclusion: This study offers new insights on the function of LIF in relation to human embryo growth and implantation, demonstrating that LIF does not affect embryo development and Bax/Bcl2 ratio but improved implantation rate. The results showed that although LIF had no effect on development rate, but it improved the expression of Klf4 gene and microRNA 145, which are involved in the growth process. It seems that it can be due to the small number of embryos and the limited availability of human embryos.
Keywords: Leukemia Inhibitory Factor (LIF), Blastocyst, Klf4

Protective Effects of Cerium Oxide Nanoparticle (CeO2 NPs) on The Human Sperm Quality Parameters After Cryopreservation

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Background: Sperm cryopreservation is a useful method for fertility preservation. However, sperm quality is affected by reactive oxygen species (ROS) production and increased oxidative stress. Cerium oxide nanoparticle (Ceo2 NPs) are able to store and release oxygen, conferring them scavenger activity against oxidative stress. This study investigated the protective effects of Ceo2 NPs on human sperm performance post thawing.
Materials and Methods: Semen samples were collected from 20 normozoospermic men. After sperm processing, each sample was divided into four aliquots: fresh, frozen without treatment and those exposed to Ceo2 NPs at the following concentrations: 0.1, 1 and 5 µg/ml. Sperm samples were mixed with Sperm Freeze Solution then loaded into a cryotube and stored for one week. The optimum concentration of Ceo2 NPs was determined by evaluation of motility parameters (computer assisted semen; CASA), viability (Eosin-nigrosine), lipid peroxidation (Malondialdehyde level: MDA) and membrane integrity (Hypo osmotic swelling test; HOST).
Results: The highest significant percentage of total motility (60.9±2.5), viability (67.9±1.5) and membrane integrity (66.7±1.85) were obtained in 0.1 µg/ ml of Ceo2 NPs, also, the lowest significant level of lipid peroxidation was observed in all Ceo2 NPs groups compared to the control group ($p<0.05$).
Conclusion: Our results showed that 0.1 µg/ ml of Ceo2 NPs could improve human sperm quality parameters after freezing-thawing. It seems that beneficial effects of CeO2 NPs for human sperm cryopreservation could be related to an intra-cellular antioxidant activity of CeO2 NPs and reduce oxidative stress level.
Keywords: Cryopreservation, Human Semen, Cerium Oxide Nanoparticle

Practical Protocol For Isolation of The Different Ovarian Cells Including Detectable Oogonial Stem Cells From Human Transsexual and Chemotherapy-Induced Secondary Premature Ovarian Failure (POF) Ovarian Tissues

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Background: The depletion of the ovarian reserve is due to the dismissal of follicular growth in chemotherapy-induced premature ovarian failure (POF). To support follicular reconstruction in chemotherapy-induced POF patients applying an artificial ovary, the transplantable artificial ovary should mimic the original organ, offering a physical (three dimensional matrix) and biological support (cells). To replicate the ovarian cell populations, this study aimed to isolate and assess the proportions of stromal and oogonial stem cells (OSC) in both POF and transsexual ovarian cortexes.
Materials and Methods: To this end, ovarian biopsies were obtained from both chemotherapy-induced POF and transsexual ovaries. The outer epithelial layer and medulla were carefully removed. The cortical tissue was cut into 5×5×5 mm3 strips and then the strips were slow-frozen. For each isolation, two pieces of frozen-thawed human ovarian cortex were finely minced and enzymatically digested (collagenase IA, 1.5 mg/ml). Finally, the cell suspension was left into a T25 culture dish with a medium of DMEM/F-12. After two passages, human ovarian cortex cells (HOCCS) were harvested for the next experiments and the isolated cells were fixed. For cell characterization, immunostaining for IFITM3 (for oogonial stem cells), Vimentin (for stroma cells) and Inhibin-α (for granulosa cells), and quantitative Real-Time PCR for IFITM3, Stella, Vimentin, FSH-R, and KI67 genes were performed.
Results: Positive cells in each staining were counted and the proportion of the different cell populations was estimated from the total number of isolated cells. Since there is no specific marker for ovarian stromal cells, we estimated the proportion of these cells by performing a Vimentin immunostaining and subtracting the proportions of OSC- and Inhibin-α-positive cells. Immunostaining showed that >80% of isolated cells were Vimentin-positive. From this pool, <5 % were OSC cells and <5% granulosa cells in both transsexual and POF ovarian cortex cells. Furthermore, in both ovarian cortex cells from transsexual and POF samples, after two passages we observed that the number of OSC had increased (>10%).
Conclusion: In conclusion, our findings approved well isolation of different detectable cells from the human ovarian pieces and showed that stromal cells represent the larger population of cells in the human ovarian cortex. Nevertheless, we were able to culture and increase the number of OSC by culture.
Keywords: Ovarian Cells, Oogonial Stem Cells, Transsexual, POF

Effects of Knockout Serum Replacement (KSR) Compared with Fetal Bovine Serum (FBS) on Growth of One-Day-Old Mouse Ovary During In Vitro Culture

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Background: In vitro culture of one-day-old mouse ovary could serve as a model for developing efficient and safe medical assisted reproductive techniques (ART) in infertile patients suffering from diminished ovarian reserves (DOR) due to various reasons such as oncotherapy. Accordingly, the present study was conducted to investigate the effect of two nutrient supplements, including fetal bovine serum (FBS) versus knockout serum replacement (KSR) on growth of one-day-old mouse ovaries during a seven-day culture period.
Materials and Methods: One-day-old mouse ovaries were cultured either with medium containing 10% FBS or with medium containing 10% KSR and samples were harvested on days three and seven of culture. Total number of follicles was assessed using hematoxylin and eosin (H&E) staining, ovarian area was measured using ImageJ software and gene expression of ki67 was evaluated by quantitative Real-Time PCR (RT-PCR).
Results: Total number of follicles did not differ between groups on day 3 ($p>0.05$), yet it was higher in ovaries cultured in KSR (384.83 ± 39.12) than FBS (247.56 ± 49.62) group on day 7

($p<0.05$). Moreover, ovarian area was not different between experimental groups on day 3 ($p>0.05$), but it was larger in ovaries cultured in KSR-containing media (166711.64 ± 6805.57 μm2) than those cultured in FBS-containing media (121179.35 ± 9959.01 μm2) on day 7 ($p<0.05$). However, gene expression of ki67 was not affected by nutrient supplement ($p>0.05$).
Conclusion: In conclusion, the present study showed that application of KSR as a nutrient supplement in medium for culturing one-day-old mouse ovary could increase the early development of follicles.
Keywords: Murine, In Vitro Ovarian Development, Knockout Serum Replacement, Fetal Bovine Serum

Difference in the in-vitro maturation potential and quality between oocytes/follicles with polycystic ovarian syndrome and normal oocytes/follicles

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Introduction & Objective: Polycystic ovary syndrome (PCOs) is one of the most common causes of infertility due to lack of ovulation. One of the current treatment methods for infertile couples with PCOs is using of in-vitro oocyte maturation method. Although immature oocytes/follicles recovered from PCOS compared to normal oocyte/follicles can be matured, fertilized, and developed in-vitro, but the implantation rate of these cleaved embryos is disappointingly low. In addition, the GDF-9 gene and BAX gene is used to evaluate the quality (GDF-9 gene) and viability (BAX gene) of oocytes and follicles in-vitro matured. This study was designed to investigate the differences in in-vitro maturation (IVM) potential, viability, and quality of both oocytes and follicles with PCOs compared to normal oocytes and follicles.
Materials and Methods: The PCOs induction in NMRI mice (n=10) was performed by intramuscular injection of 4 mg/kg estradiol valerate dissolved in 0.2mg Sesame oil once a day. After 60 days, the oocytes and follicles from normal and PCOs mice were collected, then subjected to IVM (in MEM-alpha culture medium with 10% FBS serum). After 24 hrs culture, Real Time PCR method was performed to evaluate the GDF-9 and BAX gene expression.
Results: After 24 hours of culture in the culture medium, 57.14% of the oocytes and 50% of the follicles were matured in the PCOs group. In the control group, 84.61% of the oocytes and 62.5% of the follicles were matured. Maturation percentage, viability (BAX gene expression) and quality (GDF-9 gene expression) of in-vitro matured oocytes were higher than of follicles in PCOs group ($p < 0.05$). While, the IVM rate of normal follicles in the control group was higher than normal oocytes ($p < 0.05$). Also, the comparison of the maturation rate between the control and PCOs groups showed that it was significantly decreased in the PCOs group compared to the normal group ($P< 0.05$).
Results: According to the results, the maturation rate, viability, and quality of oocytes and follicles were significantly different between the control and PCOs groups.
Keywords: PCOs, IVM, Oocyte quality, Follicle quality, Viability.

Is there difference between metabolism of oocytes and follicles with and without polycystic ovarian syndrome?

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Background: Healthy and quality of oocyte is one of the principles of fertility. Given that the culture medium is effective on nutrient utilization patterns and activities of metabolic pathways such as uptake and utilization of carbohydrates especially glucose in the developmental competence of oocytes, and glucose metabolism are also important for energy production in mammalian oocytes, One of the studies that can be done to evaluate the quality of the oocytes and follicles is to investigate the changes in the culture medium of the oocytes, especially the changes of glucose in the culture medium after maturation in-vitro. Also can be examined the differences in glucose metabolism of oocytes and follicles between normal group and polycystic ovary syndrome (PCOs) group, that the main aim of this study has been considered.
Materials and Methods: The PCOs induction in NMRI mice (n=10) was performed by intramuscular injection of 4 mg/kg estradiol valerate dissolved in 0.2mg Sesame oil once a day. After 60 days, the oocytes and follicles of normal and PCOs groups were collected, then subjected to in-vitro maturation (in MEM alpha culture medium with 10% FBS serum). High performance liquid chromatography (HPLC) method was used to study glucose changes in the culture medium. At first, the in-vitro oocytes and follicles matured were collected from the droplets of the culture medium after 24 hrs. Then the culture medium removed of each droplet to analysis of metabolite changes. The samples included maturation culture medium (MEM-alpha; control group), culture medium of matured oocytes (group I) and follicles (group II) from normal groups, and culture medium of matured oocytes (group III) and follicles (group IV) from PCOs groups (experimental groups). Polar NH2 column was used for the study of carbohydrates in this apparatus, acetonitrile with methanol as the carrier phase, deionized water as the soluble phase, and UV as detectors. 20 μl of culture medium was injected into the apparatus.
Results: After 24 hours of culture in the MEMα medium, 56.04% of the oocytes and 49.6% of the follicles were matured in the PCOs group. In the control group, 83.31% of the oocytes and 63.1% of the follicles were matured. The chromatogram was prepared for culture media (groups I-IV and control group) by HPLC device. It was observed that the rate of glucose metabolism was higher in normal group compared to PCOs group ($P< 0.05$).
Conclusion: According to the results, the rate of metabolism in the oocytes and follicles of the control and PCOs groups was significantly different that highlights it importance to analysis oocyte/follicles quality.
Keywords: PCOs, IVM, Metabolism, glucose, HPLC.

The Effect of Rapamycin on Autophagy Status in Somatic Cell Nuclear Transfer (SCNT) Embryos in Goat Species

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Background: Although somatic cell nuclear transfer (SCNT) is a promising technology, but its application is limited because of its low efficiency. Autophagy is involved in embryonic development and is necessary to destroy maternal mRNA and eliminate the accumulation of unnecessary proteins and organelles. Limited studies have shown the role of autophagy during preimplantation development in fertilized embryos, but no studies have investigated its role and status in SCNT embryos. Therefore, in this study the comparison and evaluation of autophagy status was performed between in vitro fertilization (IVF) and SCNT embryos in goat species.
Materials and Methods: Reconstructed oocytes from the SCNT process were collected 3, 6 and 9 hours after activation and then the autophagy status was evaluated by immunofluorescence staining of LC3B protein in goat species. According to studies in the IVF process, sperm penetration occurs 8 hours after insemination. In order to compare the SCNT process with IVF, IVF zygotes were collected at (8 + 3) 11, (8 + 6) 14, (8 + 9) 17 hours post insemination, fixed and immunostained similar to SCNT embryos. In addition, to evaluate the effect of rapamycin on autophagy status in SCNT embryos, the restructured oocytes were incubated in rapamycin (1, 10, 100 nM) for 6 hours and similar to IVF and SCNT embryos, they were also immunolabelled with LC3B antibody.
Results: During 6 hours after oocyte activation in SCNT embryos, no induction of autophagy was observed compared to IVF embryos, but treating the reconstructed oocytes with 10 and 100 nM rapamycin increased the LC3B protein expression (as an autophagy marker) in SCNT-derived embryos.
Conclusion: The results of our study show that autophagy is absent in SCNT derived embryos during the first 6 hours after artificial activation compared with IVF derived embryos. The first signs of autophagy were observed in SCNT embryos around 9 hours after activation. In addition, treatment of reconstructed oocytes in SCNT group with 10 and 100 nM rapamycin for 6 hours after activation induced the autophagy. These results may be a promising approach for improving SCNT efficiency through activation of autophagy with rapamycin.
Keywords: Somatic Cell Nuclear Transfer (SCNT), Autophagy, Rapamycin, LC3B

Evaluating the Effects of Different Concentrations of Human Follicular Fluid on Growth, Development, and PCNA Gene Expression of Mouse Ovarian Follicles

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Background: Follicle culture in vitro provides a method for investigating stages of folliculogenesis that can lead to preserving fertility through cryopreservation techniques. This study aims to assess the effects of various concentrations of human follicular fluid (hFF) on growth, development, and expression of the proliferating cell nuclear antigen (PCNA) gene in mouse ovarian follicles in vitro.
Materials and Methods: Preantral follicles were isolated from 14-day NMRI mouse ovaries. The follicles were cultured in basic media enriched with fetal bovine serum (FBS), follicle stimulating hormone (FSH), and insulin-transferrin-selenium, and supplemented with different concentrations of hFF (10, 20, and 30%) for 12 days. During the culture period, survival rate and follicular maturation, follicular diameter, levels of estrogen and progesterone secretion, and PCNA gene expression rate were evaluated.
Results: Survival rate, maturation, and antrum formation were significantly higher in the 10% hFF group than in the 20% and 30% hFF groups. On day 4, follicle diameter in the 10% hFF group was also higher than in the 20% and the 30% hFF group. In comparison with other groups, significantly higher estrogen and progesterone production levels were measured in the 10% hFF group. PCNA gene expression was also higher with 10% than 20% and 30% hFF concentrations.
Conclusion: The present study suggests that addition of 10% hFF to mice ovarian preantral follicle culture media enhances follicle growth and oocyte maturation.
Keywords: Human Follicular Fluid, Proliferating Cell Nuclear Antigen (PCNA), Preantral Follicle

Effect of Fenugreek Extract on Histology of Testis in Rat Fetus from Diabetic Mothers

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Background: Medicinal herbs are a rich source of natural antioxidants that are used in traditional medicine to control and treat many diseases such as diabetes. Fenugreek has long been used to control diabetes. The purpose of this study is evaluation and comparison of fenugreek extract effects on histology of fetal testis of diabetic rats.
Materials and Methods: Sixty adult female rats were divided randomly into six groups: G1 (control group) received no treatment, G2 received 5 mg/kg/day of glibenclamide, G3 received 1000 mg/kg/day of fenugreek extract, G4 received 50 mg/kg/day of streptozotocin (experimental diabetes), experimental diabetes G5 received 1000 mg/kg/day of fenugreek extract and experimental diabetes G6 received 5 mg/kg/day of glibenclamide. All six groups were being pregnant via natural mating. Afterwards, in gestational days 18th and 20th, two mother rats (in all six groups) were anesthetized, and embryos were transferred into 10% buffered formalin. After applying histological techniques, different histological parameters have been evaluated.
Results: Weight of diabetic mothers' fetuses under treatment by fenugreek and glibenclamide showed a significant decrease in comparison to diabetic control group ($p<0.05$). On contrary, number of spermatogonia, sertoli and leydig cells and seminiferous tubule diameter in 18 and 20 day embryos of diabetic and normal groups treated with the fenugreek extract and glibenclamide showed a significant increase in comparison with diabetic control ($p<0.05$).
Conclusion: This study showed that hydro-alcoholic extract of fenugreek reduces the effects of maternal hyperglycemia of diabetes mellitus due to elimination of possible damage to the structure of the male gonads.
Keywords: Fenugreek, Diabetes, Rat, Testis, Histology

Proliferation of Spermatogonial Stem Cells on Three Dimensional Nanocomposite Scaffolds

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Background: The culture of spermatogonial cells for future transplantation is necessary, because these cells play a key role in spermatogenesis process and are therefore very important. Lately, scientists use three dimensional scaffolds for culturing stem cells for simulating the testicular environment and tissue. In this study, we prepared silk scaffolds that contain Graphene oxide nanocomposite utilizing the biomimetic technique and investigated the proliferation of spermatogonial stem cells (SSCs) on them.
Materials and Methods: The identity of SSCs was confirmed by flow cytometry [ckit and GFRα1 (GDNF family receptor alpha-1)]. Spermatogonial cells were cultured and divided into 3 culture groups: (SSC + basic medium), (SSC + Silk scaffold) and (SSC + Silk / Graphene oxide nanocomposite scaffold). The Scaffolds were analyzed using scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR) to observe surface topography and the morphology. Cytotoxicity of scaffold was assayed at 24-hours, 72-hours and one week after seeding using MTT assay. The stem cells related markers for SSCs [ID4 (Inhibitor of DNA binding 4), GFRα1 and PLZF (promyelocytic leukemia zinc finger)] were detected on all experimental groups by quantitative Reverse Transcription-Polymerase chain reaction (qRT-PCR).
Results: Results showed that the expression of PLZF and ID4 in SSCs were all higher for up to 14 days for SSCs cultured on Silk / graphene oxide nanocomposite scaffold and had significantly increased than other scaffold groups ($p<0.05$).
Conclusion: In summary, we have developed a scaffold that displays in vitro biocompatibility, which may have potential use for SSCs proliferation in vitro. This three dimensional scaffold is applicable for culturing and encapsulation of SSCs.
Keywords: Spermatogonial Stem Cells, Silk Scaffold, Three Dimensional, Proliferation, Graphene Oxide Nanocomposite

Happiness and Self-Esteem in Infertile Couples Undergoing Assisted Reproductive Treatment Comparing with Oocyte and Embryo Donation Candidates

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Background: Infertility is a negative event in the life and the issue is more complex when third party reproduction is the only hope for a couple to have a child. The aim of our study is to compare happiness and self-esteem between couples undergoing assisted reproductive technology (ART) and those who are candidates for oocyte or embryo donation.
Materials and Methods: This cross-sectional study was conducted in a referral infertility center in Tehran, Iran from January 2017 to May 2018. The study sample consisted of three groups of infertile couples, candidates for oocyte donation (OD: n=38), candidates for embryo donation (ED=38), and non-candidates for third party reproduction (N: n=41). Including criteria were age at least 18 years and could read and write in Persian. Participants provided demographic and completed the Rosenberg Self-Esteem Scale and the Oxford Happiness Questionnaire. Data was analyzed by paired t test, ANOVA, using SPSS statistical software.
Results: The results showed there was any significant difference between our groups in happiness, but the mean score of happiness in men was higher than women in three groups ($p=0.042$). Self-esteem in ED ($p=0.015$) was significantly lower than the other two groups and in ED group self-esteem was also significantly lower in wives than husbands ($p=0.035$). The mean score of happiness was significantly higher in husbands than wives in OD group ($p=0.003$).
Conclusion: In this study we found that in general, embryo and oocyte donation candidates group have less happiness and self-esteem than normal infertile couples and need more psychological counseling for these groups.
Keywords: Happiness, Self-Esteem, Infertility, Embryo Donation, Oocyte Donation

Impact of cAMP Analog and IBMX on Sperm Quality in Sperm Cryopreservation in Asthenozoospermic Men

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Background: To explore whether the addition of cyclic adenosine monophosphate (cAMP) analog and inhibitor phosphodiesterase (IBMX) after cryopreservation improves the recovery of sperm quality and what role cAMP plays in this recovery.
Materials and Methods: Asthenozoospermic semen samples that were collected from 50 volunteers were processed. The seminal plasma was incubated with cAMP analog at 0 (control), 2.5, 12.5 and 25 mM and 0.2 mM IBMX for 180 minutes before freezing spermatozoa. After 2 weeks these samples were quick-thawed. Sperm parameters, viability, acrosome reaction and DNA damage levels were evaluated.
Results: The added cAMP analog and IBMX showed especially significant difference from the control samples. The presence of cAMP of 12.5 and 25 mM and 0.2 mM IBMX at 180 min, showed sperm motility and viability improvement ($p<0.05$). Acrosome reaction in high concentration of cAMP analog increased compared to control group ($p<0.05$). DNA fragmentation in 25 mM and 0.2 mM IBMX at 180 min of cAMP analog incubation was significantly lower than other groups ($p<0.05$).
Conclusion: In vitro cAMP analog and IBMX supplementation during asthenozoospermic samples freezing is a useful cryo protectant for increasing recovery of motile and viable sperms, and acrosome reaction and decreasing DNA fragmentation.
Keywords: cAMP Analog, Cryopreservation, Asthenozoosper-

mic, Sperm Quality

Effects of Resveratrol on the Rooster Semen Quality Characteristics After Freeze-Thaw

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Background: Semen cryopreservation is one of the most important reproductive techniques in livestock and poultry industry. However, cryopreservation causes death of a significant proportion of sperms. Using antioxidants decreases levels of reactive oxygen species (ROS) and consequently improves sperm parameters after freeze-thaw. This study investigated the protective potential of resveratrol (RSV) on rooster sperm function post thawing.
Materials and Methods: Semen samples were collected twice a week from ten roosters. Then samples were pooled and divided into four equal parts to be diluted with Beltsville extender containing different concentrations of RSV as following groups: 0 (control), 0.01, 0.1 and 1 µm. Diluted semen was gradually cooled to 4 °C for 3 h. Then cooled semen was loaded into 0.5-mL straws and stored for one week. The optimum concentration of RSV was determined by evaluation of motility parameters (computer assisted semen; CASA), viability (Eosin-nigrosine stain) and membrane integrity (Hypo osmotic swelling test; HOST).
Results: The highest significant percentage of total motility (60.9±2.6), viability (57.9±1.5) and membrane integrity (60.7±2.3) were obtained in 0.1 µm RSV and 1µm RSV produced the lowest significant percentage of motility (30.8±2.6), viability (40.7±1.5) and membrane integrity (40.4±2.3) compared to the other groups ($p<0.05$).
Conclusion: Our results showed 0.1 µm of RSV could improve rooster quality parameters after freezing-thawing. These results could be relevant for implementing prospective changes in the optimization of rooster sperm cryopreservation protocol.
Keywords: Cryopreservation, Rooster Sperm, Resveratrol

Evaluation of IWR1 Effect, An Inhibitor of WNT Signaling Pathway, on Pre-Implantation Development of IVF and SCNT Derived Embryos in Goat Species

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Background: Despite many advances in improving the in vitro fertilization (IVF) technique, its efficiency in terms of post-implantation development is lower compared to natural pregnancy in different species. There are three main messenger pathways involved in embryonic development: WNT, FGF and TGF-β. Among these pathways, the WNT pathway plays an important role in developmental processes. There is limited information about the role of WNT pathway on development of fertilized embryos. Previous studies have shown that activation of WNT pathway during post compaction stages can reduce the pre-implantation development. Regard to this, in this study we assessed the effect of WNT inhibition using a small molecule (IWR1) during post compaction stages on pre-implantation development of IVF embryos in goat species.
Materials and Methods: In order to evaluate the inhibition of WNT pathway on blastocyst rate of IVF embryos, 3 concentrations of IWR1 (1.25, 2.5, 5 µM) were added to culture medium 4 days after fertilization. The blastocyst rate was assessed on day 7 of in vitro development. In addition, total cell number (TCN), inner cell mass number (ICM) and trophectoderm number (TE) were evaluated through differential staining in derived blastocysts from various treated groups.
Results: Our results showed that treatment of IVF derived embryos with 1.25 and 5 µM IWR1 significantly increased the blastocyst rate (32.25±3.58 % and 33.69±5.64 %, respectively) compared to the control group (20.38±2.68 %). The number of ICM, TE and TCN did not change following the treatment with IWR1 in blastocyst embryos.
Conclusion: The results of our study showed that inhibition of WNT pathway with an efficient small molecule, IWR1, can improve pre-implantation embryonic development in terms of blastocyst rate.
Keywords: WNT Signaling Pathway, SCNT, IWR1 Inhibitor

Photobiomodulation Preconditioned Human Semen Protects Sperm Cells Against Detrimental Effects of Cryopreservation

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Background: The biological consequences of preconditioning of semen samples by photobiomodulation (PBM) on cryopreserving human sperms were studied.
Materials and Methods: Donated semen samples were collected from 22 married men with normal sperm parameters according to World Health Organization (WHO) criteria. Included samples were divided into control and PBM-preconditioning (one session, 810 nm, diode laser, and 0.6 J/cm2) groups before cryopreservation procedure. Progressive sperm motility (PSM) and morphology, viability, sperm mitochondrial membrane potential, intracellular reactive oxygen species (ROS) and lipid peroxidation of sperm cells were assessed post thawing.
Results: PBM preconditioning of cryopreserved semen samples compared to control ones showed significant increases in the PSM percentage 30, 60 and 90 minutes post thawing (respectively, $p=0.000$, $p=0.026$, $p=0.004$). The number of viable spermatozoa post thawing increased significantly ($p=0.000$) by application of PBM before cryopreservation. The number of spermatozoa depicting high membrane potential after freezing-thawing in control group was very low (10.84 ± 1.33). Application of PBM before cryopreservation significantly ($p=0.004$) increased the number of spermatozoa with high mitochondrial activity. Meanwhile, PBM therapy decreased significantly the intracellular ROS level of cryopreserved human sperm cells (47.66 ± 2.14) compared to the control ones (60.42 ± 3.16) (t-

test, p=0.002). Preconditioned PBM group showed a significant decrease in the level of lipid peroxidation (3.06± 0.13) compared to the control cryopreserved group (3.68 ± 0.27) (p=0.05).
Conclusion: Our findings, as the first evidence, indicated that PBM-preconditioning of human semen before cryopreservation provides a real and substantial advantage. This might lead to a novel strategy in improving PBM application in the ART procedures.
Keywords: Photobiomodulation, Cryopreservation Procedure, Sperm Motility, Intracellular Reactive Oxygen Species, Sperm

Effect of Trehalose on Human Spermatozoa Freeze-Drying

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Background: Freeze-drying is one of the sperm preservation methods leading to preserve, sperm genetic material for a period up to 1.5 years in 4˚C. In this method, different stresses have detrimental effects like sperm membrane and loss of sperm motility on spermatozoa. In order to reduce these damages and improvement of sperm parameters, the effect of trehalose, as a cryoprotectant agent, was evaluated during the freeze-drying process.
Materials and Methods: Twenty-five normozoospermic semen samples were included in this prospective study. The sperm parameters were assessed before and after swim-up. The samples were divided into two groups of control (without trehalose) and trehalose. The freeze-dried samples were stored at 4˚C. The sperm parameters including count, motility, morphology, viability, acrosome reaction, DNA denaturation and fragmentation were evaluated before and after freeze drying.
Results: The sperm parameters were significantly reduced after freeze-drying compared to before this process. Sperm viability, acrosome integrity and DNA integrity showed increased trend after freeze-drying in trehalose group compared to control. However, viability, acrosome reaction and non-denatured sperm DNA was significantly higher in trehalose group in comparison with control group. The results showed that the freeze-drying caused immobilization in spermatozoa. Freeze-drying reduced normal morphology and addition of trehalose did not affect this parameter.
Conclusion: The results of this study showed trehalose can prevent the detrimental effects of freeze-drying on sperm parameters.
Keywords: Freeze-Drying, Sperm, Trehalose, DNA Integrity

Bioactive Peptides Attenuates Heat Induced Degenerative Effects in The Rat Testis

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Background: Heat stress in testicular tissue, even for a short time, has the ability to destroy testicular tissue and disrupt spermatogenesis. Bioactive peptides with their antioxidant activity play an important role in the metabolic function of organisms and human health. The aim of this study was to evaluate the antioxidant effects of bioactive peptides on heat stress induced damage in rat testis.
Materials and Methods: Fifty-six male Wistar rats were randomly divided into 8 groups including: 1- control, 2- bioactive peptide, 3, 4 and 5- heat stressed (37, 39 and 43 °C water bath, 20 min), 6, 7 and 8- heat stressed along with bioactive peptides (10 mg/kg; oral gavage), respectively. The animals were sacrificed 35 days after heat treatment and their testes were taken for quantitative Real-Time-PCR and immunohistochemical analysis.
Results: Heat stress increased testicular tissue damage, germ cells apoptosis, Bax mRNA and protein expression, reduced Bcl2 mRNA and protein expression. Treatment with bioactive peptide as a substance with antioxidant properties ameliorated the damage caused by heat stress.
Conclusion: The results of this study highlight the protective role of bioactive peptides in reproductive tract under heat stress and its potential function against testicular tissue as well as in oxidative stress, apoptosis and apoptosis inducer genes expression reduction.
Keywords: Heat stress, Bioactive peptide, Apoptosis, Rat, Testis

Genetics

P-111: New Players in Repeated Implantation Failure: The Effect of Endometrial Scratching on Endometrial Status by Modulating Innate and Adaptive Signaling Pathway Genes Expression

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Background: Endometrial scratching (ES) prior to embryo transfer in the IVF cycle in women with the repeated implantation failure (RIF) has been suggested repeatedly, but the exact molecular mechanisms underlying scratching-induced improvement of the endometrium has not been presented till now.
Materials and Methods: Fourteen patients with unexplained RIF who failed to achieve pregnancy after three or more IVF/ICSI cycles and top-quality embryo transfer (ET) included in this randomized controlled trial (RCT) study. After informed consent, patients were randomly allocated to either the ES group (endometrial biopsy during the proliferative and luteal phases of the cycle preceding ovarian stimulation, n=7) or the non-ES group (biopsies in the luteal phase prior to stimulation cycle, n=7). The cDNA from samples was used for RT2 Profler PCR Array according to the manufacturer's protocol (innate and adaptive immune response kit, PAHS-052A, Qiagen). This kit array consists of 84 genes related to the human innate and adaptive immune responses genes.
Results: As expected, there were differential expression level of numerous genes which induced by endometrial scratching between ES group and non-ES group. These genes involved in T-cell activation, adaptive and humoral immunity, T helper 1 (Th1), Th2 and Th17 immune response markers, T-regulatory marker and pattern recognition receptors.
Conclusion: Endometrial scratching in unexplained RIF patients may exert positive effects on the implantation by modulate immune signaling pathway. The assessment of the molecular network patterns, especially certain genes in innate and adaptive immune responses in such patients could improve the strategies for dealing with the reasons for their infertility.
Keywords: Endometrial Scratching, Repeated Implantation Failure, Innate and Adaptive Immune Response

P-112: Concentration of Testosterone in Mares Carrying Male and Female Fetuses over Various Stages of Pregnancy

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Background: Sex determination of the fetus is of importance in equine industry due to economic reasons. Conventionally, sex determination of fetus is implemented using transrectal ultrasonographic examination of the mare between days 60 to 80 of gestation, which is a limited period and necessitates of an alternative method that could be performed during further stages of pregnancy. In this context, evaluation of maternal testosterone concentration has been indicated as a measure for sex determination of bovine fetus; however, no study, to the best of our knowledge, investigated this method in equine.
Materials and Methods: To understand whether circulating concentration of testosterone differ between mares carrying male and female fetuses, blood samples were collected from mares (n = 20) at months three, six and nine of pregnancy, and subsequently, concentration of testosterone was measured using a chemiluminescence immunoassay kit. The gender of foals was determined at the time of parturition.
Results: Concentration of testosterone did not differ between mares with male fetuses (n = 11; 60.07 ± 5.12 pg/ml) and mares with female fetuses (n = 9; 51.86 ± 4.96 pg/ml). However, testosterone concentration was higher at month six of pregnancy (81.20 ± 4.39 pg/ml) as compared with months three (31.00 ± 10.35 pg/ml) and nine (45.90 ± 3.06 pg/ml) of pregnancy.
Conclusion: In conclusion, the present study showed that maternal testosterone concentration could not be used for sex determination of fetus in horse. Yet the current study revealed dynamics of testosterone concentration over various stages of gestation in mares.
Keywords: Equine, Testosterone Concentration, Sex Determination of Fetus , ,

P-113: Rutin Effect on Sirtuin-3 Gene Expression on BALB/C Mice Ovary Tissue

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Background: Women's lifestyle has changed in recent years and daily stress has increased their fertility problems. Sirtuin-3 (sirt3) is expressed in mitochondria and has a great role in reactive oxygen stress (ROS) deactivation. Rutin is a bioflavonoid that has anti-inflammatory effects. Also, it has anti-cancer effects especially in large intestinal, colorectal and liver cancers. Rutin reduces tumor size and increases cancer cell death and is effective in treating polycystic ovaries. In the current research, the effect of Rutin on the ovarian tissue and the expression of sirt3 gene was investigated.
Materials and Methods: Twelve adult female mice of BALB/C were divided in two groups: control group without any injection and test group that received as intraperitoneal injection (IP) 150 mg/kg of Rutin that solved in solvent (DMSO+Tween80+saline) every other day for two weeks. Then ovarian tissue sections were stained with Hematoxylin-Eosin. Quantitate evaluation of the sirt3 gene expression has been done with the quantitative Real-Time PCR method and the data were analyzed with REST software.
Results: According to the tissue results Rutin has a stimulating effect on the ovary and increased the number of follicles. Rutin also led to significant increase in the expression of sirt3 gene in ovarian tissue ($P<0.05$).
Conclusion: Rutin has a stimulating effect on the folliculogenesis process through sirt3 activation. Sirt3 upregulation decreases the ROS level in the cell and protects it from harmful

factors. Rutin, can be under attention in treatment of fertility related disorders.
Keywords: Rutin, Sirtuin, Ovary

P-114: The Effect of Cytarabine on BALB/C Mice Ovary and Sirtuin-3 Gene Expression

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Background: Due to increasing risk of infertility during chemotherapy, recognizing the effects of chemotherapy drugs is necessary. Sirtuin-3 (sirt3) gene is expressed in the mitochondria of cells and has a vital role in granulosa cells and human cumulus cells during folliculogenesis, luteinization, aging process, progesterone regulation and reactive oxygen stress (ROS) deactivation. Also, its role in oocyte fertilization and fetal development has been proven. In the current research the effect of anticancer antimetabolite drug, Cytarabine, is investigated on ovarian tissue and sirt3 gene expression.
Materials and Methods: Twelve adult female mice of BALB/C, weighing 25 to 30 gr were divided in two groups: control group with no injection and test group that received 100mg/kg of Cytarabine in the form of Intraperitoneal injection (IP) in single dose. After two weeks, ovarian tissue sections were stained with Hematoxylin-Eosin. Quantitate evaluation of the sirt3 gene expression has been done with the quantitative Real-Time PCR method and the data were analyzed with REST software.
Results: Cytarabine destroyed the structure of ovarian tissue and reduced all types of follicles. Cytarabine also led to significant decrease in the expression of sirt3 gene in ovarian tissue ($P<0.05$).
Conclusion: As the reduction of sirt3 expression significantly increases the ROS level of the cell and subsequently destroys DNA, RNA, proteins, lipids and other cell components the reduced expression of sirt3 in ovary tissue should be considered in Cytarabine application.
Keywords: Cytarabine, Sirtuin, Ovary

P-115: The Effect of Prenatal Lead Acetate and Electromagnetic Field on The Expression of Psd-95 Gene in The Hippocampus of 17 Day Fetal Mice

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Background: Lead acetate is a heavy and highly toxic metal known as a destructive factor in the developmental stages, which can lead to decreased neurogenesis, learning and memory disorders. Nowadays, electromagnetic stimulation of the brain has a wide range of therapeutic applications due to its significant effect on neurogenesis, proliferation and survival of neurons, synaptogenesis and vascularization. In this study, the expression of Psd-95 gene in the hippocampus of 17-day-old fetuses of mice that used lead acetate throughout pregnancy and exposed to electromagnetic waves was examined by quantitative Real-Time PCR method.
Materials and Methods: Male and female adult mice (NMRI) were placed in cages for mating. By observing vaginal plaque in female animals, day 0 of pregnancy was identified and randomly divided into 4 groups as follows: Sham group: pregnant mice that were placed in an electromagnetic device without receiving waves and distilled water (twice daily), from day 0 of pregnancy; Lead acetate group: pregnant mice fed 1cc of lead acetate (5 mg / kg, twice daily), from day 0 of pregnancy; Electromagnetic field (EMF) group: pregnant mice were exposed to EMF (intensity 2 mT and frequency 50 Hz, 4 hours daily). EMF and lead acetate group: pregnant mice exposed to EMF and fed lead acetate at the same time. Pregnant animals were sacrificed on day 17 and the extracted hippocampal samples obtained from embryos assessed by quantitative Real-Time PCR method.
Results: Using Tukey complementary test, there was a significant decrease in the relative expression of Psd-95 gene in the lead treated group compared to sham. There was also a significant decrease in the relative expression of Psd-95 gene in the EMF treated group compared to sham. While in the lead + EMF treated group, no significant difference was observed in the relative expression of Psd-95 gene compared to the lead group.
Conclusion: Exposure to lead and EMF in sensitive embryonic period by affecting expression of Psd-95 gene can affect hippocampus development. It can also affect neurogenesis.
Keywords: Lead, Electromagnetic Field, Hippocampus, Psd-95, Pregnant Mice

P-116: Aberrant Chromatin Incorporation of Peroxisome Proliferator-Activated Receptor γ on Promoters of Fatty Acid Metabolism Genes in Testis Tissues of Men with Impaired Spermatogenesis

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Background: Fatty acid metabolism genes contribute to a normal spermatogenetic process. Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor that is activated by ligands and subsequently regulates the genes expression of Fatty Acid Desaturase 2 (FADS2), Fatty Acid Elongase 2 (ELOVL2) and Stearoyl-CoA desaturase (SCD1) in testis tissue during spermatogenesis. This study aimed to investigate the

incorporation of PPARγ on promoters of the mentioned fatty acids metabolism genes in infertile men.
Materials and Methods: In this case-control study, the cases groups were included 10 men with maturation arrest (MA) and 10 men with Sertoli cell-only syndrome (SCOS), and the control group was included 10 men with obstructive azoospermia (OA) which have normal spermatogenesis. Chromatin Immunoprecipitation (ChIP) coupled with quantitative Real-Time PCR was used to determine the presence of PPARγ transcription factor on the promoter regions of the FADS2, SCD1, and ELOVL2 genes. All data were analyzed using One-Way ANOVA.
Results: The incorporation of PPARγ on promoters of FADS2, SCD1, and ELOVL2 genes was significantly reduced in SCOS and MA groups in comparison with the OA group ($P<0.05$). However, there was no significant difference between the two groups with defective spermatogenesis (MA and SCOS).
Conclusion: Poor incorporation of PPARγ on promoter regions of FADS2, SCD1, and ELOVL2 genes reflect the reduced expression of the mentioned fatty acid metabolism genes in testis tissues of men with impaired sper**matogenesis which cause to male infertility/subfertility.**
Keywords: Epigenetics, Male Infertility, Fatty Acid Metabolism, Peroxisome Proliferator-Activated Receptor γ (PPARγ)

P-117: Differential Expression of Fatty Acid Metabolism Genes in Testis Tissues of Men with Normal and Impaired Spermatogenesis

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Background: The genes involved in fatty acid metabolism can influence spermatogenesis. Fatty acid desaturase 2 (FADS2) and fatty acid elongase 2 (ELOVL2) play important roles in fatty acid biosynthesis by converting the essential dietary fatty acids into poly-unsaturated fatty acids (PUFA). Also, Stearoyl-CoA desaturase (SCD1) has a critical role in the formation of monounsaturated fatty acids (MUFAs). The current study investigates the expression profile of the aforementioned fatty acid metabolism genes in the testicular biopsies of infertile men.
Materials and Methods: In this case-control study, the cases groups were included 10 men with maturation arrest (MA) and 10 men with Sertoli cell-only syndrome (SCOS), and the control group was included 10 men with obstructive azoospermia (OA) which have normal spermatogenesis. Testes tissues were obtained through Microsurgical Testicular Sperm Extraction (Micro-TESE). RNA extraction and cDNA synthesis were done on collected samples and mRNA levels of FADS2, ELOVL2, and SCD genes were evaluated by quantitatively real-time polymerase chain reaction (real-time PCR). The data were analyzed using one-way ANOVA.
Results: Based on the results, the expression levels of FADS2 and ELOVL2 were significantly lower in MA and SCOS than the control group (OA). Also, the expression of SCD1 was significantly decreased in SCOS rather than the OA group ($P\leq0.05$). However, there was no significant difference between the two groups with defective spermatogenesis (MA and SCOS).
Conclusion: It seems that decreased expression of FADS2, ELOVL2, and SCD1 have destructive effects on sperm motility and function, which consequently cause male infertility/subfertility.

P-118: The Effect of Melatonin and L-Carnitine on GDF-9 And BMP-15 Gene Expression in Culture Condition of Mouse Oocyte

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Background: Melatonin and L-carnitine are free radical scavengers, anti-apoptotic and antioxidant agents that contribute to improvement of oocyte development. The aim of this study was to evaluate the possible effects of combination of these two antioxidant agents on oocyte morphology, maturation, apoptosis and the expression levels of GDF-9 and BMP-15 genes in a mice model.
Materials and Methods: Over stimulation was done in 60 female NMRI mice using intreperitoneal injection of mare serum gonadotropin (PMSG). On 24 hours post-injection, oocytes were obtained from each mouse. The harvested oocytes were randomly divided in four groups including control and three treatment groups: melatonin and L-carnitine alone and combination of melatonin and L-carnitine. The morphology, maturation rate and apoptosis of the oocytes were then evaluated using a light microscope and TUNEL assay. Then, gene expression of BMP-15 and GDF-9 was measured by quantitative Real-Time PCR technique.
Results: Combination therapy with of melatonin and L-carnitine increased oocyte diameter ($p\leq 0.003$). The highest mean percentage of oocyte cytoplasmic pattern was detected in the melatonin+ L-carnitine group. Results of TUNEL test indicated that apoptosis rate was the lowest in the group. The obtained data from gene expression showed that both BMP-15 and GDF-9 were significantly up-regulated in the any treatment groups. Moreover, the highest number of oocytes and maturation rate were observed in the melatonin group.
Conclusion: Our results indicated that a significant promotion in oocyte maturity in the all treatment groups compared to control group. The highest maturity rate and gene expression were detected in the melatonin+ L-carnitine group. Although there were almost no significant differences between combination therapy and treatment with either melatonin or L-carnitine,

these results are in favor of co-administration of melatonin and L-carnitine as a more effective choice for *in vitro* promotion of oocyte maturation.
Keywords: *In Vitro* Maturation (IVM), Melatonin, L-Carnitine, Oocyte, BMP-15, GDF-9

P-119: Time Dependently Effects of Reperfusion on Expressions of Transcriptional Factors SOX2, Oct4, Nanog in Spermatogonial Stem Cells in Torsion-Induced Rats

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Background: Testicular torsion (TT) is one of the most important urological problems, which negatively affects the spermatogenesis process. The only suggested therapeutic approach is the testicular reperfusion (TR) surgery to ameliorate the damages. One of the main pluripotency/self-renewal mediators of spermatogonial stem cells (SSCs) is Sox2 protein, in association with Oct4, as the target gene of Sox2, results in the Sox2-Oct4 complex, which initiates the SSCs self-renewal process. The Nanog mainly expresses in SSCs during stage XII of spermatogenesis, resulting in SSCs subpopulation self-renewal. Thus, the present study was conducted to study the time-dependently effects of TR on transcriptional factors Sox2, Oct4, and Nanog expressions after the TT induction.
Materials and Methods: To follow up, 30 mature Wistar rats were randomly divided into control and experimental groups (N=6 in each group). The experimental group animals sub-divided into 2 hours unilateral right testis torsion-induction and TR-induced groups (1, 2- and 4-hours post-TR). Following 2 hours TT, the testicular samples were dissected out from six rats of the TT group, and the other rats had undergone TR. Following test termination, the protein levels of Sox2, Oct4, and Nanog were evaluated using immunohistochemistry (IHC) and the positive cell number was analyzed by Image J software.
Results: The animals in TT and TR-induced groups, represented a diminished expression of Nanog, enhanced levels of Sox2, Oct4 versus the control group. Moreover, the numbers of Sox2+, Oct4+, and Nanog+ cells per mm2 were increased and decreased, respectively. In more detail, T2R4 groups of animals illustrated a significant up-regulation of Nanog protein levels versus T2, T2R1, and T2R2 groups.
Conclusion: Considering the important role of Nanog protein in SSCs self-renewal process, it could be concluded that 4 hours post-TR surgery is the minimal time to re-initiation the self-renewal process of SSCs.
Keywords: Torsion, Reperfusion, Time-Dependently, Self-Renewal

P-120: Epigenetic Modifications in DMR Region of H19 Gene in Endometrial Tissues of Women with Endometriosis

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Background: Endometriosis is defined by the presence of endometrial-like tissue outside of the uterine cavity, which has been considered an epigenetic disease. The long non-coding RNA (lncRNA) H19 and insulin-like growth factor-2 (IGF2) genes form a reciprocally imprinted cluster (IGF2/H19). The regulatory sequences of expression of these two genes include imprinting control region (ICR). The ICR region is located between the two genes and is a differentially methylated region (DMR). IGF2 and H19 genes play important roles in regulating cellular growth and differentiation and might be targeted by methyl-DNA binding protein MeCP2 (as a marker of DNA methylation) for subsequent epigenetic modifications through the DMR regulatory region.
Materials and Methods: In this case-control study, 10 endometrial samples (eutopic) and 10 endometriotic lesions (ectopic) of women with endometriosis and 10 endometrial control samples were analyzed. Control samples were obtained from women who had no evidence of endometriosis during diagnostic laparoscopy. Control and eutopic endometrial samples were obtained by pipelle. Ectopic samples were obtained during laparoscopy. All women signed the informed consent form and did not receive any hormonal treatments during the last three months. Parallel to analysis the gene expression profile of H19 and IGF2 genes by quantitative Real-Time PCR, the occupancy of MeCP2 on DMR region of H19 gene was investigated using chromatin immunoprecipitation (ChIP) technique.
Results: Although the expression levels of H19 and IGF2 were significantly decreased in eutopic and ectopic endometrial lesions compared with control group, the ChIP Real-Time PCR data revealed no significant difference in DMR DNA methylation profile between the monitored eutopic, ectopic and control samples.
Conclusion: It seems that the DNA methylation profile of DMR region in IGF2/H19 is not associated with the gene expression profile of IGF2 and H19 during endometriosis, and maybe other epigenetic players are involved in this regard.
Keywords: Endometriosis, Epigenetic, DMR Region, H19

P-121: Evaluation of Single Nucleotide Variants in Exon 5 of AURKC Gene in Aneuploid Aborted Fetuses

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Background: Infertility is a global public health issue. One of the main factors in pregnancy loss as an infertility related problem is chromosome segregation errors. For proper germ cell production, all the factors involved in the cell cycle should have properly function during meiosis. Protein kinase AURKC is an essential subunit of spindle assembly checkpoint (SAC) complex.
Materials and Methods: We collected products of conception from mothers under the age of 36 to rule out advanced maternal age as a contributing factor in aneuploidy incidence. Quantitative fluorescence polymerase chain reaction (QF-PCR) and/or array comparative genomic hybridization (aCGH) methods were used to diagnose fetal aneuploidy. We investigated single nucleotide variants (SNVs) in exon 5 of AURKC gene certainly a pathogenic SNV, rs397515484, in 50 aneuploid aborted fetuses. We used PCR and Sanger sequencing methods then data was analyzed using FinchTV software.
Results: There was no evidence of heterozygote and/or homozygote variant in exon 5 of AURKC gene in 50 studied samples.
Conclusion: rs397515484 does not seem to be incident in aneuploid fetuses, therefore screening of couples with a history of abortion for this SNV is not a priority.
Keywords: Miscarriage, Aneuploidy, AURKC Gene, Single Nucleotide Variant (SNV), rs397515484

P-122: Differential Expression of DYRK1 Gene in Testis Tissues of Men with Normal and Impaired Spermatogenesis

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Background: In the process of producing healthy sperm, there are several signaling pathways including Hedgehog (Hh) playing crucial roles regarding to cellular differentiation processes in testis. In mammals, the Hh signaling pathway regulates the proliferation and differentiation of spermatogonial stem cells (SSCs) in direct and indirect ways. A protein kinase called DYRK1B is known as a negative and positive regulator of Hh signaling in different cell types. DYRK1B gene is mainly expressed in skeletal muscle and testis and is detected at lower levels in most other normal tissues. In this case-control study we compared the mRNA expression of DYRK1B in the testicular biopsies of infertile men referred to Royan Institute.
Materials and Methods: The patients enrolled in the study were included 10 men with maturation arrest (MA) and 10 men with Sertoli cell-only syndrome (SCOS) as cases groups with impaired spermatogenesis, and the control group was included 10 men with obstructive azoospermia (OA) which have normal spermatogenesis. Testes tissues were obtained through Micro-surgical Testicular Sperm Extraction (Micro-TESE). RNA extraction and cDNA synthesis were done on biopsies samples and the expression levels of DYRK1B gene were measured in the 3 sample groups by quantitative Real-Time PCR.
Results: Our results demonstrated a significant decrease ($P<0.05$) in DYRK1B gene expression in cases groups in comparison with control group.
Conclusion: It is suggested that lower expression of DYRK1B can be considered as a genetic factor for male infertility/subfertility.
Keywords: Male Infertility, DYRK1B, Gene Expression

P-123: The High Fat Diet Reduces Proliferating Cell Nuclear Antigen (PCNA) Expression and Amplifies The Heat Shock Proteins Expression; An Experimental Study

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Background: Obesity, a lifestyle syndrome, induce oxidative stress, hormonal dysregulation, and metabolic disruption in testicular tissue eventually suppressing spermatogenesis. The Heat Shock (Hsps) and proliferating cell nuclear antigen (PCNA) proteins, aside from their homeostatic interactions under endocrine and cellular stress conditions, actively protect the DNA and RNA integrities during replication phase in the germ cells. Therefore, here in the present study, we have investigated the obesity-induced impact against Hsps (90 and 70) and PCNA expression to find out the possible mechanism for severe DNA and RNA damage in obese condition.
Materials and Methods: To induce obesity, the rats received high fat diet (HFD, n=10) and 10 Rats with basic diet were considered as control group. After 8 weeks, the samples were collected, processed and Hematoxylin and Eosin staining was done for analyzing the histomorphometry of the testis tissue. The Leydig cells steroidogenesis and serum testosterone were assessed by fluorescent staining and ELISA techniques, respectively. The Hsp70-2a, Hsp90, and PCNA expression was assessed. The special fluorescent staining was assigned to detect the mRNA damage, and DNA integrity was assessed by DNA laddering test.
Results: The serum testosterone and the steroidogenic potential of Leydig cells were decreased in the HFD-received animals with no changes in Leydig cells distribution/mm^2 of interstitial tissue. The animals in the HFD group represented a significant ($P<0.05$) up regulation in the Hsp70-2a and Hsp90 expression. However, the PCNA expression was remarkably decreased in

the HFD group compared to the control rats. Finally, the obesity induced sever mRNA and DNA damage at germ and somatic cell levels.
Conclusion: We can conclude that due to the suppressing effect of obesity on PCNA expression, the compensatory increased Hsp70-2a and Hsp90 do not protect DNA and mRNA integrity.
Keywords: Obesity, Heat Shock Protein, PCNA, Spermatogenesis

P-124: Evaluation of Oxidative Phosphorylations Genes Expression in Cumulus Cells of The Patients with Polycystic Ovary Syndrome

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Background: The relationship between oxidative stress (OS), insulin resistance (IR) and polycystic ovary syndrome (PCOS) is an important medical issue in human reproduction. Cumulus cells (CCs) remain in close contact with the egg even after ovulation. To the best of our knowledge there is no report on oxidative phosphorylation genes expression in CCs of PCOS however some of the genes of interest has been previously studied in granulosa and muscle cells of these patients.
Materials and Methods: Twenty-four women <36 years under GnRH antagonist protocol were studied in PCOS-IR, PCOS-OS, based on insulin resistance/sensitivity, and male factor (control) groups. Expression of 10 OXPHOS genes including NCF2, TXNIP, CYC1, NDUFB6, NDUFA3, SDHA, SDHB, COX7C, ATP5PD and UCP2 was studied by quantitative Real-Time PCR and normalization was performed considering HPRT1 expression as a tissue specific reference gene. Kruskal-Wallis and Mann-Whitney were used for data analyses. All statistical tests were two-sided and $P<0.05$ was considered statistically significant.
Results: NCF2 expression in IR group was significantly higher than control and OS groups ($P=0.003$) and TXNIP upregulated in IR in comparison with controls ($P=0.042$). Overexpression of CYC1 gene in IR was marginal ($P=0.07$). The expression of the other seven OXPHOS genes was not significantly different between groups.
Conclusion: Overexpression of NCF2 and TXNIP genes is in accordance with previous findings in granulosa cells of PCOS. These results suggest that OXPHOS genes alteration can be considered as a potential molecular scenario for the pathophysiology of PCOS.
Keywords: Polycystic Ovary Syndrome, Cumulus Cells, OXPHOS Genes Expression

P-125: Effective Co-Expression Networks in Recurrent Implantation Failure

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Background: Recurrent implantation failure (RIF) refers to failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. The causes of RIF are varied, especially due to various maternal and paternal factors that affect the genetic and morphological status of the fetus. Network theory allows for a holistic understanding of the role of genes in diseases. No studies have been performed on co-expression networks of this disease. The purpose of this study is to identify identification of hub genes and gene modules that that can cause the RIF.
Materials and Methods: In this study, data from fifteen endometrial tissue samples of RIF patients were downloaded from GEO database. The expression data is normalized by RMA algorithm and Affymetrix expression software console. The differentially expressed genes (DEGs) were identified by t-test and then the WGCNA R software package was applied to construct the co-expression network based on the expression data profile of DEGs. Genes function was annotated based on Gene Ontology the DAVID.
Results: In this study, several gene modules were identified, one of which was involved in embryo development and the other in innate immune system. A number of gene hubs such as FBXL19-AS1, IFNA14 and OR51B5 were also identified in these modules.
Conclusion: WGCNA was able to identify significant groups of genes associated with RIF such as genes involved in the immune system and embryo excretion and embryo development.
Keywords: Recurrent Implantation Failure, WGCNA, System Biology, Co-Expression Network

P-126: Investigation of Single Nucleotide Variations in The Exon 8 of SYCP3 Gene in Aneuploid Aborted Fetuses

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Background: Chromosomal abnormality is one of the most common causes of spontaneous abortion. Aneuploidy could be associated with maternal age. Synaptonemal complex protein 3 (SYCP3) is a protein scaffold that is formed between homologous chromosomes during the prophase in meiosis. A 4-base pair deletion in the exon 8 of SYCP3 gene in women with recurrent pregnancy loss has been previously reported. The aim of this study was to evaluate the probably association of single nucleotide variations (SNVs) in the exon 8 of SYCP3 certainly the aforementioned deletion, c.553-21_553-18del (rs587776620), with aneuploidy in aborted fetuses.
Materials and Methods: To rule out the effect of advanced maternal age on aneuploidy occurrence, 40 aneuploid products of conception was collected from mothers <36 years that their aneuploidy was confirmed by quantitative fluorescence

polymerase chain reaction (QF-PCR) and/or array comparative genomic hybridization (aCGH). We analyzed the exon 8 sequence using PCR Sanger sequencing and the results were analyzed with FinchTV software.
Results: No variant was observed in 40 studied samples for the exon 8 of SYCP3 gene.
Conclusion: Since the rs587776620 variant was not observed in 40 studied samples, this variant does not seem to be frequent in aneuploid aborted fetuses so as its examination is not prioritized for couples with history of aneuploid fetuses and their embryos.
Keywords: Aneuploidy, SYCP3 Gene, Miscarriage, Single Nucleotide Variant (SNV)

P-127: Evaluation of The ALX1 and PDHX Genes Expression in Endometrial Tissues of Women with Endometriosis in Comparison with The Control Group

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Background: Endometriosis is a benign, estrogen-dependent disease and the leading cause of infertility in women of reproductive age. This disease is characterized by the presence of endometrial glandular tissue and stroma outside the uterus. Endometriosis is considered as a multifactor disease affected by genetic, hormonal and environmental factors. Among genetic factors, Aristaless-like homeobox1 (ALX1) and Pyruvate Dehydrogenase Protein X (PDHX) genes are considered in this study. Studies show that increasing expression of the ALX1 gene cause to increase in cell proliferation, migration and invasion in cancer cells. Another candidate gene, PDHX, is involved in cellular metabolism, in the way that effectively functions as a tumor suppressor gene by maintaining normal metabolic homeostasis. Till now, the specific roles of the ALX1 and PDHX in endometriosis remain unclear. In this study, we investigated the expression of the ALX1 and PDHX in endometrial tissue of women with endometriosis in compare to control group.
Materials and Methods: In this experimental study, five normal women and five women with endometriosis were enrolled. Ectopic biopsies were obtained with the use of laparoscopy, while the endometrial control samples (as a control group) and Eutopic samples were collected via pipelle. RNA extraction and cDNA synthesis were done on collected samples and quantitative Real-Time PCR technique was used for gene expression analysis.
Results: Our preliminary data showed that gene expression levels of ALX1 and PDHX were higher in ectopic and utopic endometrium of women with endometriosis in comparison to control group.
Conclusion: It is suggested that deregulation of ALX1 and PDHX genes could be involved in pathogenesis of endometriosis.
Keywords: Endometriosis, ALX1, PDHX, Gene Expression

P-128: Gene Expression of CREB Binding Protein in Endometrial Tissues of Women with Endometriosis

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Background: Endometriosis is defined as presence and growth of endometrial tissues outsides of uterine cavity. Hormonal dysregulation and immune imbalance have been involved in pathogenesis of endometriosis. Autoimmune regulator (AIRE) as one of transcription factors regulates expression of tissue specific antigens via its partners. It has been reported that AIRE expression is changed in endometriosis. One of AIRE partners is CREB binding protein (CBP) which acetylates AIRE leading to its repression. In this study, we investigated gene expression of CBP in endometrial tissues of women with endometriosis in compare to controls.
Materials and Methods: Eleven women with endometriosis (endometriosis group) and fourteen women without endometriosis (control group) were enrolled after diagnostic laparoscopy in this case control study. Eutopic endometrial tissues of endometriosis and control groups were taken by pipelle. Ectopic endometrial samples were collected from women with endometriosis during laparoscopy. RNA extraction and cDNA synthesis were done. Quantitative Real-Time PCR was used for gene expression analysis. GAPDH gene was used as housekeeping gene.
Results: Results showed that CBP gene expression was increased in eutopic and ectopic endometrial tissues of women with endometriosis in compare to controls. Although these increases were not statistically significant.
Conclusion: It seems overexpression of CBP gene in endometrial tissues of endometriosis may be involved in its pathogenesis.
Keywords: Endometriosis, CREB Binding Protein (CBP), Gene Expression

A Systems Biology Approach to Gene Expression Analysis Between Triple Negative Breast Cancer and Metastatic Hepatocellular Carcinoma

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Background: Triple negative breast cancer (TNBC) is a type of breast cancer in which estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER-2) are not expressed. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults, and is the most common cause of death in people with cirrhosis. Cancer from other organs also may spread to the liver. Other common sites are the lung, and brain. In this study we find the important and common genes that are therapeutic targets in these two diseases.
Materials and Method: genes related to TNBC and HCC were extracted from Genecard database. Then Common genes of these two diseases were identified by venn diagram. The network was constructed by Cytoscape software (version 3.5.1). Then main component of the network was analyzed considering centrality parameters including degree, betweenness, closeness and stress. Furthermore, Gene Ontology (GO) analysis of the key genes was performed.
Results: Output of genecards database shown 4076 genes in TNBC and 7392 genes in HCC are differentially expressed. 2828 genes were common between TNBC and HCC. EGFR, MYC, ALB, VEGFA, TNF, MAPK3, SRC, STAT3, FN1, MAPK1, HRAS, MAPK8 are the genes with high degree among the genes respectively. 12 crucial genes were analyzed by GO analysis, among all, biological Process “regulation of macromolecules” (GO:0010604), was disclosed as top category followed by Molecular Function” Map kinase activity” (GO:0004707).
Conclusion: The analysis of common genes of the TNBC and HCC showed that there are some common crucial genes including EGFR, MYC, ALB, VEGFA, TNF, MAPK3, SRC, STAT3, FN1, MAPK1, HRAS and MAPK8 which are tightly related to progress of disease. Therefore, these genes and pathways can be considered as an appropriate target for control and treatment of TNBC and HCC.
Keywords: Hepatocellular carcinoma, TNBC, Gene ontology

Ethics and Health

P-129: Prevalence of Sexual Dysfunction among The Most Common Causes of Infertility.

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Background: Sexuality as a vital part of women's health, can be affected by infertility. This study focuses on comparing the prevalence of sexual dysfunction among women with the most common causes of infertility and providing the prevalence of sexual dysfunction.
Materials and Methods: This cross-sectional study was performed between May 2016 and June 2017 on 240 infertile females (at Royan Institute) and 160 fertile women (at health care centers). Infertile women consisted of three groups of women with polycystic ovary syndrome (PCOS, n=80), endometriosis (n=80) and male factor (n=80). Sexual function was assessed by Female Sexual Function Index (FSFI). Data were analyzed with SPSS (version 25.00). Differences were regarded statistically significant for P<0. 05.
Results: The prevalence of female sexual dysfunction in women with PCOS, endometriosis and those with male factor infertility were 98.8, 100.0 and 80.0% respectively. In total, 36.2% of the participated fertile women were suffering from sexual dysfunction.
Conclusion: There was a relationship between the prevalence of female sexual dysfunction and infertility etiologies. Therefore, infertility care providers are necessary to draw attention to this problem and develop preventive strategies in this regard.
Keywords: Infertility, Female Sexual Function, Prevalence

Reproductive Imaging

P-130:The Relationship between Sonographic Markers of Ovarian Morphology and Serum Testosterone Levels in Hirsute Women with Regular or Irregular Menstrual Cycles

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Background: Biochemical assessments of androgens in women are associated with inconsistent or challenging results. Male pattern hair growth is the most common clinical manifestation of elevated androgens levels in women. There is growing evidence elucidating a significant role for ovarian sonography in the assessment of androgen excess. In this study, our purpose was focus on the relationship between total serum testosterone levels, ultrasound finding of ovaries and menstrual patterns (regular versus irregular menstruation cycle) in hirsute women.
Materials and Methods: It is a bicentric case control study in two affiliated university hospitals. Ninety three reproductive age (18 – 45 years) women with male pattern hair growth (according to modified Ferriman- Gallwey scoring system) were evaluated for their menstrual pattern, total serum testosterone level (liquid chromatography spectrometry), and ovarian morphology (transvaginal ultrasound).Regional and total modified Ferriman- Gallwey (mFG) score system, ovarian volume (OV), stroma to ovarian area ratio (S/A), stromal echogenicity index (SI) ,number of follicles in each follicle size category, follicle numbers in each ovary (FNO), total serum testosterone (TST), and menstrual regularity were compared.
Results: There is no correlation between total mFG score and TST in women with and without regular menstrual cycles (P=0.04). A statistically significant relationship between TST and hirsutism score in lower abdominal and thigh was seen in women with regular menstruation (P=0.03) and, a statistically significant relationship between TST and hirsutism score in thigh, lower abdominal, and upper arm areas were detected (P<0.02). The sonographic marker FNO of follicles <6 mm was able to predict TST in women with irregular menstruation (P=0.01).
Conclusion: Total mFG could not reflect TST levels. In contrast, in the subgroup of hirsute women with irregular menstruation, the numbers of follicles <6 mm have ability to predict TST levels.
Keywords: Hirsutism, Testosterone, Ferriman- Gallwey Score System, Sonography

P-131:Relationship Between Diameter of Amniotic Sludge and Preterm Labor

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Background: Prevalence of preterm labor (PTL) is 5-18%. Subclinical intra uterine infection is an important factor in the PTL. Amniotic sludge is an indicator of subclinical infection is seen as a suspended hyper echo mass, near cervix. The aim of this study was to assess the relationship between the diameter of amniotic sludge and the preterm labor in the pregnant women referred to ultrasound ward of Royan Institute.
Materials and Methods: This retrospective study was carried out on the eligible pregnant women referred to ultrasound ward of Royan institute, Iran in 2017-2018. The women who had amniotic sludge in their ultrasound examination were selected. The diameter of the amniotic sludge and gestational age (GA) at delivery was determined. GA less than 37 weeks was considered as PTL; accordingly cases were divided into two groups. Data were entered to SPSS 21 software and p value less than 0.05 was considered significant.
Results: Overall, in 75 cases with the amniotic fluid sludge the prevalence of PTL was 33.3%. Since 15 cases had twin pregnancy, they were omitted. The prevalence of PTL was 20% after omitting the twin pregnancies as a risk factor of PTL. Mean cervical length was significantly lower in PTL group (P=0.003), but there were no significant difference between diameter of sludge in the groups (P=0.11). After omitting cases with cervical length less than 30 mm, 36 cases were remained. The prevalence of PTL was 8.3% after omitting the cervical length less than 30 mm. Using Mann-Whitney test, there were no significant difference between diameter of sludge in the groups (P=0.1).
Conclusion: The prevalence of PTL in pregnant women with amniotic fluid sludge is higher than general population. It seems that the sludge affects the cervical length and causes PTL. There is no significant relationship between the diameter of amniotic sludge and the preterm labor. Further study with control group and larger sample size is recommended.
Keywords: Preterm Labor, Sludge, Cervical Length, Ultrasound

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Abstracts of

Royan International Twin Congress

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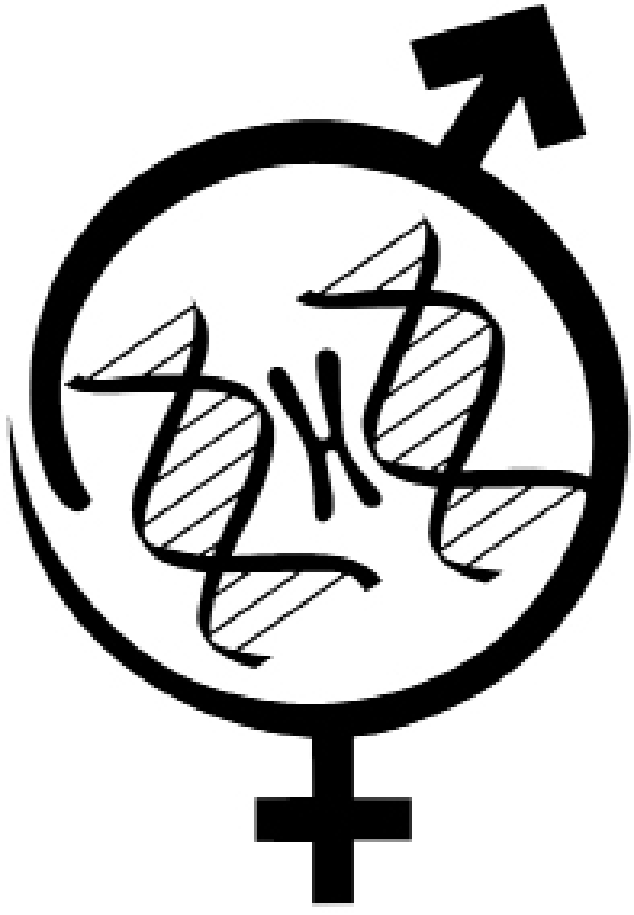

Royan Institute

Reproductive Biomedicine Research Center

Tehran, Islamic Republic of Iran

Inm-1: The Role of Nurses and Midwives in POF Counseling and Treatments

Ahmadi M

Inm-2: The Role of Genetic Factors in POF

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Premature ovarian failure (POF) is one of the major causes of female infertility owing to an abnormal ovarian reserve ,characterized by amenorrhea, hypoestrogenism, and elevated gonadotropin levels in women under the age of 40. Primary ovarian insufficiency is. Its relevance has increased in more recent years due to the fact that age of motherhood is being delayed in developed countries, with the risk of having either primary ovarian insufficiency or less chances of pregnancy when women consider the option of having their first baby. POI is a heterogeneous disease caused by a variety of mechanisms. Though the underlying cause remains unexplained in the majority of cases, various data indicate that POI has a strong genetic component. Genetic factors are the most commonly identified cause and the impact of sex chromosome abnormalities (e.g., Turner syndrome or X structural abnormalities), autosomal and X-linked mutations on the genesis of primary ovarian insufficiency has also been well described. Yet in most cases, the genetic origin remains unknown and there are multiple candidate genes. This review aims to collect all the genetic abnormalities and genes associated with syndromic and non syndromic primary ovarian insufficiency. The unbiased approaches of genome-wide association studies and next-generation sequencing technologies have identified several novel genes implicated in POF. However, owing to POF's diverse etiology and genetic heterogeneity, we expect to see the contribution of several new and novel molecular pathways that will greatly enhance our understanding of the regulation of ovarian function. A better understanding of POI pathogenesis will indeed allow the construction of tests able to predict the age of menopause in women at higher risk of POI. The identification of several causative genes may allow for early detection and would provide better opportunity for early intervention, and furthermore, the identification of specific gene defects will help direct potential targets for future treatment.

Inm-3: Redefining Stress from Quran's Perspective

Chitchian H
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Inm-4: Pelvic Diseases, Pelvic and Ovarian Surgeries, Oncology and Treatment in POF

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POF is a challenging issue as women are delaying having families and this emotionally distressing problem must be dealt , on both the physical and psychological platform. POF is a condition characterized by amenorrhea, hypoesterogenism, and elevated serum gonadotropin levels in women younger than 40 years. Incidence: 1-4 % of women .Etiology: Idiopathic (spontaneous) or Iatrogenic, usually unknown. Consequences: Short term: vascular symptom (hot flash, night sweats), Headaches, Vaginal dryness, Dyspareunia, Urge and Stress urinary incontinency, Irritability, Forgetfulness, Poor concentration. Long Term: Infertility, Osteoporosis, Cardiovascular disease, Stroke, Psychological Impact.
Management: HRT (Hormone replacement therapy): Cyclic HRT or continuously HRT, Estrogen (Transdermal,). Progesterone. Androgen.
ART: Oocyte donation, Embryo donation, Ovarian Cryopreservation in Iatrogenic POF. New Treatment: Stem cell Therapy,Adoption
Management and Consolation: Endocrinologist consolation, Psychological evaluation and consoling,Genetic consoling, Management and Lifestyle Modification: Diet: Elemental Calcium, Vitamin D. Activity: Weight bearing exercises, outdoor Sports.

Inm-5: The Role of Training in Reducing Couples' Stress

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Infertility is a biop-sychosocial condition and it may be difficult for students to understandwhat infertile individuals experience. There are different assisted reproductive techniques in the treatment of infertility.
Anxiety and stress have been shown to be markedly higher in infertile couples.
Stress makes many body organs work harder than normal and increases the production of some important chemicals in your body, including hormones.
Education and counselling is one of the effective methods for reducing the perceived stress in the women undergoing assisted reproductive treatment.Relaxation technique is another methods that reduces the stress and can balance the human's emotions.
The following tools have been demonstrated stress reducer's. Aerobic exercise, walking, yoga, and relaxation training all have an ability to improve not only physical health but psychological well-being as well. Activities that help produce relaxed states such as guided imagery journaling and mind-body groups are also well known stress reducer's.
Thus Education is possible to help them so they can help you.

Inm-6: The Role of Clinician in Initial Encounter with Infertile Couples

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Infertility is described in failure to achieve child after one year. Couples after encounter to this problem, experience negative consequences of childlessness. They should solve social, economic and medical problems. In some countries infertility is a cultural problems too.
Nurses and midwives are the first members of infertility center to manage infertile couples. So, their role is too critical in center. They should achieve counseling skill to their client and pay attention to emotional and psychological effect of infertility to couples. Infertility is long life crisis for couples and their family, they may experience many failure cycles, abortions or other medical problems. So, they should care and manage the couples in all times during treatment. Also, nurses and midwives must learn consultation skill to give "breaking bad news".

Inm-7: The Role of Positive Thinking in Reducing Couples' Stress

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There is a lot of research on the effects of stress on infertility today. Although a direct link between the two has not yet been confirmed, stress is still one of the factors influencing infertility. One of the most fundamental questions is whether infertility causes stress or stress causes infertility? Although the main findings of the research are in favor of the first question, regarding stress coping strategies, the difference between stress and anxiety should be considered because it plays an essential role in creating stress management strategies in infertile couples. Gender will also be effective in applying stress management strategies due to the fact that women and men respond differently. Although stress management strategies are strongly influenced by people's views and attitudes, but can be observed in general principles, the most important of which is positivism. Positivism is a process that starts from mental conflict and ends in self-worth. The input of this process is the mental conflict between infertility stress and the desire for self-efficacy. Mental activities include: identifying virtues, controlling guilt, creating golden questions, how to document events, etc., which ultimately leads to self-worth, not blaming others, not leaving daily activities, asking for help from others in a timely manner and etc.

Inm-8: The Immunologic Reasons of POF

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Immune system in partnership with autonomic nervous and vascular endothelial cells help to maintain of whole body homeostasis. Tissue development and regeneration could be controlled by cytokines and growth factors derived from immune system cells. Immune cells and their secreted factors are participated in proliferation and differentiation of ovarian germline stem cells that differentiate to ovarian germ cells and primary granulosa cells. Degeneration of ovarian committed resident immune cells that are recited in embryonic time could alter ovarian function, regeneration of ovarian follicles and gamet mitotic activity and result in premature ovarian failure.
Disregulation of immune system can influence the maintenance of different organs. Autoimmunity could affect ovaries as well as other organs. This could be seen in polyglandular autoimmune syndromes that could be along with non-endocrine autoimmune diseases. At least two endocrine organ dysfunction like type I diabetes, autoimmune thyropathy, Addison's disease, hypothyroidisms, hypogonadism should be found in this syndrom. Some other autoimmune premature ovarian failure is associated with non-glandular autoimmune diseases as it could be in individuals with ANA and anti-phospholipid antibodies. In some studies the association of anti-TPO and premature ovarian failure were observed.

Pnm-1: Practical Personalized Medicine: Key Choice into Prevention Drug-Drug Interactions

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Background: In the past decade, our understanding of the genetic factors influencing response to a variety of drugs has been considerably expanded. It seems, fast-growing advances in the molecular genetic technology pave our way to enter Right drug, right dose road of the pharmacological treatment. This approach, coupled with growing interest in safe-effective Treatment (SET), led to a major shift in the clinical practice to the practical personalized medicine.
Materials and Methods: The personalized medicine may be a cornerstone of effective pharmacological treatment and prevention of adverse effect associated with pharmacotherapy and significant harm associated drug interactions. Do not make mistake, personalized medicine is not a Magic wand. All know, the all drugs have multiple effects with only a limited number of identifying ones. Also, in parallel with, there is a safe diversity in the mankind, that well-known as polymorphism. Please stop, there is a main question. What is personalized medicine in practice: a luxury issue vs. profound concept or a complex, sophisticated clinical practice vs. precise but simple clinical practice.
Results: The personalized medicine provides insight into establishing effective and powerful treatment. Among this, there is a major trend toward incurable or hard curable diseases, particularly breast cancer. Growing interest in personalized medicine, coupled with the falling price of sequencing, has been developed the demand for ordering genetic tests by a physician in this type of diseases. At the first view, it sounds so good. But there are some challenges such as disabling and expensive chronic disorders and drug-drug interactions, e.g. migraine and statin therapy, respectively. There is clearly a need to pay more attention and avoid of misunderstood and mismanaged. Indeed, there was a significantly higher risk of adverse drug reactions (ADRs), for example approximately 197,000 deaths annually in Europe.
Conclusion: In other words, these are some simple basic points that, unfortunately, neglect of these, is yet accompanied with an undesirable consequence, that interfering with daily life, affecting lifespan and causing social disability. It seems, pay more attention to the drugs taken before drug therapy may be a key parameter, that, may provide a key choice into prevention drug-drug interactions, such as statin therapy. The sufficiently strong nature of personalized medicine may influence a wide range of diseases and/or drug therapy. In reality, however, it is more important to have a precise and fair look at it and develop its principle application to clinical medicine. The personalized medicine is not a luxury industry or exclusive to cancer. Also, it is not always, a complex, sophisticated clinical practice.
Keywords: Practical Personalized Medicine, Drug-Drug Interactions, Clinical Practice

Pnm-2: Does Sexual Function Differ between Infertile Women with Polycystic Ovary Syndrome and Endometriosis?

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Background: The aim of this study is to evaluate and compare sexual function and prevalence of sexual dysfunction in infertile women with polycystic ovary syndrome (PCOS) and endometriosis.
Materials and Methods: This cross-sectional study was performed on 420 infertile women with endometriosis and PCOS (210 in each group) and 210 healthy women of reproductive age as a control group between 2018 and 2019 referred to the Infertility Clinic of Arash Hospital in Tehran, Iran. Sexual function was assessed by the female sexual function index (FSFI). One-way ANOVA and logistic regression were used to analyze the data.
Results: The mean total score of FSFI in the endometriosis group was significantly lower than PCOS and control groups and the mean total score of FSFI in the PCOS group was significantly lower than the control group ($P<0.001$). In the endometriosis group, the score of orgasm, pain, and satisfaction subscales were significantly lower than PCOS and control groups. In the PCOS group, the score of desire, arousal, and lubrication subscales was significantly lower than the endometriosis and control groups ($P<0.001$). Logistic regression analysis between Socio-demographic variables and the probability of endometriosis and PCOS indicated that educational status and occupation were predictive variables ($P<0.001$).
Conclusion: The rate of sexual dysfunction is high in infertile women with endometriosis and PCOS, so it is necessary to pay attention to this issue by infertility care providers in infertility centers.
Keywords: Polycystic Ovarian Syndrome, Endometriosis, Infertility, Sexual Function

Pnm-4: Evaluation of Fertility Factors Related to Fast-Foods Consumption Based Nutritional Impacts

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Background: Heavy metals have been identified as factors affecting human fertility. Chemical contaminants are widespread throughout male exposure is virtually unavoidable. Aims of this study were to provide firsthand data on the incidence of trace metals in human seminal plasma and find possible correlations between levels of toxic metals and semen quality of Iranian population.
Materials and Methods: This review article summarizes recent literature assessing preconception dietary intake and the association with fertility and also the relationship with nutritional impacts; these conditions associate with each other, and also with infertility. The impact of paternal diet is also reported.
Results: Many studies concluded that many fast foods com-

posed of them were rich in energy compared with the nutrients they offered. If customers are to select nutritious meals from these menus, effective nutrition education programs are needed.
Conclusion: The present systematic review of observational studies provides the most comprehensive analysis to date of the associations between diet or nutrient intake and the risk of male infertility.
Keywords: Fast Foods, Fertility, Nutrition, Reproductive

Pnm-5: Aging and Reproductive Performance

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Background: In the past few decades there has been a noticeable trend among women in many parts of the world to delay childbearing until relatively late into their reproductive years, and the first time mothers who are over the age of 30 has increased steadily. One of the factors that may contribute to this trend is a general lack of knowledge about the decline in fertility with age.
Materials and Methods: This review article has been extracted from 28 articles that has indexed in most valid scientific cites that has published from year 2015 to 2020.
Results: In recent years women are having fewer children and they are delaying births to a later age than in previous centuries while decreased fecundity with increasing female age has long been recognized from demographic and epidemiological studies. Age- related decline in fertility (at around age 30) involve several factors; germ cells in the female are not replenished during life, attrition and utilization of follicles leads to a decline in the number of oocytes from birth to menopause, the quality of existing oocytes diminishes with age and an average intercourse frequency declines with age. In addition, pregnant women at an advanced maternal age also face an increased risk of pregnancy complications such as primary caesarean delivery, prolonged and dysfunctional labor, pregnancy hypertension, and delivery before 32 weeks. It is possible that this gap of knowledge is due to health care programs. Likely have an educational emphasis on pregnancy prevention rather than the infertility awareness.
Conclusion: Our finding suggests that there is a lack of knowledge concerning the decrease of infertility with age and there is a need for education on fertility and aging particularly among some high- risk groups.
Keywords: Women, Reproductive, Age

Pnm-6: Alleviate Risk Factors of Tubal Infertility

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Background: Infertility is an increasingly significant health problem in many areas of the world. Tubal pathology is one of the most frequent causes of female infertility. The incidence of tubal infertility varies between 30 and 40% whilst successful treatment of tubal infertility is limited. The prevalence of tubal infection was significantly dependent on the number of lifetime sexual partners thus education can be helpful.
Materials and Methods: This review article has been extracted from 28 articles that has indexed in valid scientific cites that has published from year 2016 to 2020.
Results: Tubal pathology, judged severe enough to be responsible for the patient's infertility alone. Presumably the increasing incidence of infertility is consequent to an increasing incidence of tubal infection with N. gonorrhoeae, C. trachomatis, or other sexually transmitted pathogens. Chlamydia trachomatis (CT) infection is a common sexually transmitted disease. CT infections often remain asymptomatic, and therefore undetected. It has been suggested that CT, together with Neisseria gonorrhoeae, is the most common cause of tubal factor infertility. The prevalence of CT infection was significantly dependent on the number of lifetime sexual partners. Condom use was inconsistent, and lack of knowledge about CT infections and associated health risks. The data indicated a need for health education concerning CT to be targeted at female adolescents.
Conclusion: Primary care services recommended yearly chlamydia screening for those adolescent female considered to be at risk. Chlamydia screening in primary care detects a large burden of asymptomatic infection in a variety of demographically defined populations.
Keywords: Infertility, Chlamydia Trachomatis, Health Risks, Sexually Transmitted Disease

Pnm-7: Nutrition and Risk of Endometriosis in Infertile Women

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Background: Endometriosis is a chronic and common disease in the world in which glands, stroma and endometrium are found outside the uterine cavity. The presence of this tissue in different parts of the body causes various symptoms that adversely affect women's quality of life, fertility and work productivity. In this study, we aimed to investigate the relationship between risk factors and nutrition with endometriosis.
Materials and Methods: This study is case-control and was performed in all infertile women who underwent laparoscopy in two groups, case group (with diagnosis of endometriosis with pathology) and control group (without endometriosis). The sample size is estimated to be 125 in each group. Data collection tools were demographic information questionnaire and nutrition questions questionnaire using standard lifestyle questionnaire (LSQ). Data analysis was done using SPSS software version 20. Quantitative variables were used using t-test and Chi-square test was used to compare qualitative variables.
Results: There was a direct and significant relationship between the risk of endometriosis and age, education level and regularity of menstruation variable so that with the increase of the above variables, the probability of disease increases. There was a significant and inverse association between the incidence of endometriosis and the body mass index (BMI) variable.
Conclusion: This study suggests that high age, menstrual cycle regularity, low BMI and education have strong relationship with endometriosis in infertile women and there was no relationship between nutrition and endometriosis in infertile women.
Keywords: Endometriosis, Nutrition, Infertility, Laparoscopy

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