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

15th 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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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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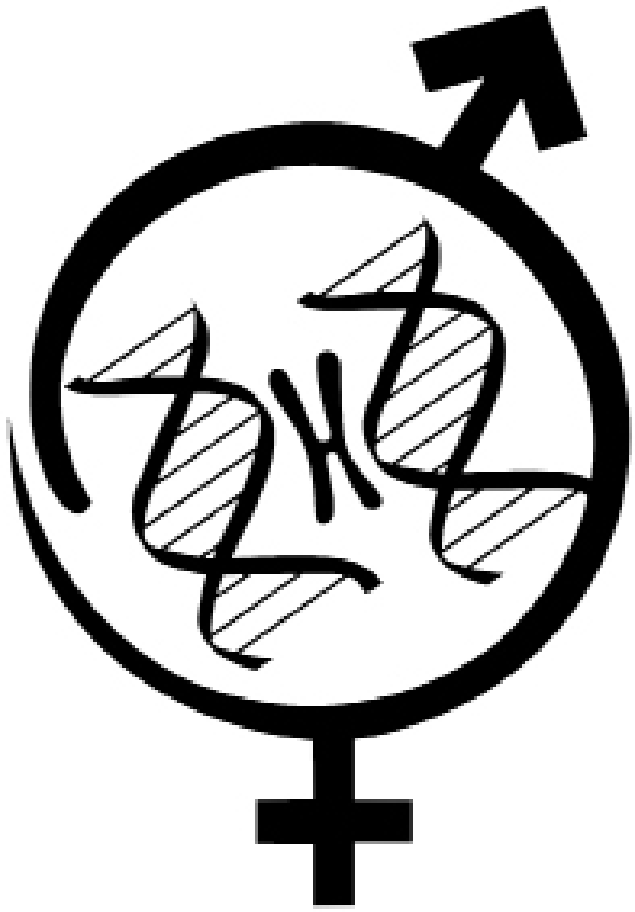

Royan Institute

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

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

Embryology

P-29: Effect of Myoinositol Supplement on the Quality of Frozen- Thawed Human Sperm on Patients with Oligoasthenoteratozoospermia Syndrome

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Background: Oligoasthenoteratozoospermic syndrome is common presentation in male infertility. this syndrome occurs by decreasing motility, numbers and morphologic changes sperm. The production of oxygen species increases, in the semen abnormal. then it may affect motility, morphology and DNA stability of sperm. This study aimed at evaluating the effect of Myoinositol in human semen parameters and DNA fragmentation patients with syndrome after thawing procedure.
Materials and Methods: Semen samples were obtained from 40 oligoasthenotetratozoospermia patients, aged 28-45, which have been referred to IVF center of Akbar Abadi hospital. Semen samples were collected through masturbation into sterile containers, following 2–4 days sexual abstinence. All the samples after by CASA were immediately in two groups, group 1: (2 mg per ml) + freezing medium (Life Global), group 2: freezing medium. Samples were thawed. Semen parameters in two groups were analyzed by CASA for motility and morphology. Also, the level of total ROS, TAC, DNA fragmentation and MDA were evaluated by DCFHby Fluorimetry), ELISA kit, TUNEL assay by flow cytometry and ELISA respectively.
Results: Our data clearly showed that total, progressive motility were higher in the experimental group. Also, DNA fragmentation is lower in myoinositol than control (22.4% vs. 29.6%, p0>001). TAC is higher in myoinositol than control (1.12mM vs. 0.95 mM, P>0.05) and MDA is significantly decreased in myoinositol than control (2.1 nmol vs. 2.3 nmol, P<0.05). The level of ROS was decreased in myoinositol group but not significant (179.4 mM vs. 200.8mM, P<0.05).
Conclusion: These data suggest that improves sperm parameters through the mitochondrial mechanism and it can play a protective role sperm DNA and increases TAC capacity. So, This study showed that can be used as a supplement in sperm freezing process in patients with Oligoasthenoteratozoospermia syndrome.
Keywords: Myoinositol, Sperm Cryopreservation, DNA Damage, Oligoasthenoteratozoospermi Syndrome

P-30 Increasing Pregnancy Rate in Mice Using Vaginal Cytology in Different Phases of Estrus Cycle in Mice

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Background: Determining different stages of the estrus cycle is an effective method for tracking various stages of the cycle, as well as selection of mature mice that are ready for pregnancy or pseudo-pregnancy. Determining different phases of the estrus cycle is possible by examining the changes in vaginal smear epithelial cells, which is not only quick and inexpensive, but also one of the most accurate methods for identifying various stages of mouse estrus cycle. The stages of estrus period were determined according to the proportions and morphology of leukocytes and epithelial cells. In the proestrus dominance stage only nucleated cells are observed. The estrus phase consists of the Cornified cells. The next stage, Metestrus, represent the same percentage of Cornified cells, nucleated cells and leukocytes. Finally, in the diestrus stage leukocytes are dominant.
Materials and Methods: In order to prepare sterile specimens from mouse vagina, physiological sterile sodium chloride (0.9% NaCl) was used and the samples were stained using 0.1% crystal violet dye. After vaginal smear and determination of estrus cycle stages, the mice were mated individually for with vasectomized male mice overnight, and the mice vaginal plaque was observed and recorded the following morning.
Results: 30 ten weeks old mice were selected, of which 11 mice were in the Proestrus and Estrus cycle. Of these 11 mice, 81.8% were positive for plaque and ready for mating. The rest mice were in the Metestrus and Diestrus cycle, of which only 2 (10.5%) mice showed positive plaque, i.e. ready for mating.
Conclusion: Mice that are in the Proestrus and Estrus stages are ready to mate and after mating, pregnancy or pseudopregnancy is achievable. Therefore, the proestrus and estrus cycle, can be considered as the best time for embryo transfer to recipient mice.
Keywords: Pregnancy, Mice, Vaginal Cytology, Estrus Cycle

P-31: Protective Effects of Silymarin on Spermatogenesis Parameters following Testicular Torsion-Detorsion in Mouse

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Background: Testicular torsion (TT) is an emergency and acute event that occurs most often in infants and adolescents, and if not treated promptly, it often leads to infertility. Detorsion results the blood reperfusion and ultimately more damage occurs to the testicle. Silymarin is a polyphenolic flavonoid that plays an important role in the improvement of many diseases due to its antioxidant properties. The aim of this study was to investigate the histological changes of the testis in mice that are affected by unilateral testicular torsion-detorsion (TD) and also to evaluate the protective effects of silymarin on the damage induced by TD.
Materials and Methods: In this study, 32 adult male NMRI mice were randomly divided into four groups. Group 1: control sham, Group 2: silymarin, in two groups without testicular torsion application, Group 3: TD, and Group 4: TD plus silymarin (TD+S). The testicular torsion was performed by rotating the 720° spermatic cord the left testis in a counterclockwise direction. After 1 hour, with a rotation opposite to the previous direction, Detorsion was done. After the detorsion, the animals received 50 mg / kg of silymarin via gavage for 35 days. At the end of experimental, All of the left testicles were removed. After fixation in 10% formal saline, paraffin sections were prepared and stained with hematoxylin-eosin. Histomorphometri-

cal studies were performed to evaluate the spermatogenesis process. Data were analyzed by ANOVA and post hoc Tukey test ($P < 0.05$).
Results: The mean number of spermatogonia, primary spermatocytes, rounded and elongated Spermatide in TD groups were reduced in comparison to the other groups. Silymarin in TD+S group significantly ameliorated spermatogenesis parameters compared to the TD group ($P<0.05$).
Conclusion: The results showed that spermatogenesis parameters improved with the administration of silymarin. This may be due to the antioxidant effects of silymarin.
Keywords: Silymarin, Spermatogenesis, Torsion, Testis

P-32: Beneficial Effect of Platelet-Rich-Plasma on Testicular Ischemia-Reperfusion Injury in Adult Mice

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Background: Testicular torsion (TT) is a common syndrome that could lead to function loss and infertility in the testis. Ischemia due to testicular torsion and reperfusion related to the detorsion can cause different morphological changes in testicular tissue. In addition, reperfusion induces increased tissue damage after ischemia. Therefore, testicular ischemia and subsequent reperfusion (I/R) can lead to testicular tissue damage. Platelet Rich-Plasma (PRP) may has therapeutic effect due to the presence of growth factors. In this study, PRP was used to evaluate its effects.
Materials and Methods: Thirty adult male NMRI mice were randomly divided into three equal groups. The first group, is the healthy control, without testicular twisting. Group II: ischemia - reperfusion (I/R) + Pbs (phosphate-buffer-solution) group, Group III: The I/R + PRP group. In these two groups spermatic cord were twisted for one hour, and after detorsion, Pbs and PRP were injected into the rete testis, respectively. 35 days after surgery, left testis sampled for histomorphometrical examinations. After fixation in 10% formal saline, paraffin sections were prepared and stained with hematoxylin-eosin. Obtained data were statistically compared by ANOVA and post hoc-Tukey test ($P < 0.05$).
Results: Cross-sectional area, the number of tubules and numerical density of the seminiferous tubules were significantly decreased in the I/R-Pbs group compared to the other groups ($P < 0.05$). I/R caused a significant increase in interstitial space of testicular tissue in I/R group compared to the control and I/R+ PRP groups ($P<0.05$). PRP could reduce these tissue changes compared to the I/R group($P<0.05$).
Conclusion: PRP is effective for the prevention of testicular torsion damage in mice testis. It seems that, PRP due to possession ample of growth factors, capable to relatively improve the undesirable effects of I/R.
Keywords: Ischemia, Reperfusion, Platelet-Rich-Plasma, Testis, Torsion

P-33: Positive Effects of Bioactive Peptides on Sperm Viability and Motility in Male Rate Treated with Cimetidine

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Background: Cimetidine is widely used for digestive tract ulcers but induces testis injury. Bioactive peptides are extracted from protein sources and a number of studies have suggested as a beneficial agent during peroxidative damage. In this study positive effects of Bioactive peptides on sperm viability and motility in male rate treated with cimetidine was investigated.
Materials and Methods: Forty- two male rats (165 ± 20 g) were randomly divided into six groups of seven animals and were fed with treatments (Control, peptides 20 mg/kg, cimetidine 40mg/kg, cimetidine 120mg/kg, peptides+ 40 mg/kg cimetidine, peptides + 120 mg/kg cimetidine) for 6 weeks. At the end of the experiment, all the animals were anesthetized with chloroform, the vase deferans separated. The eosin-negrosine dyeing and Neubauer lams were used to determine sperm viability and motility respectively.
Results: The results showed that dietary treatments significantly affected on viability of sperm and the highest value was observed in the animals fed bioactive peptides treatments which had significant difference with cimetidine treated rats ($P<0.05$). No significant difference was seen in sperm motility in animals ($P>0.05$).
Conclusion: Based on our results, boactive peptides extracted from marine sources improve viability of sperm in cimetidine treated rats but did not affect the sperm motility. Aforementioned results can be explained according to bioactive peptides antioxidant activity which reported in several references.
Keywords: Cimetidine, Bioactive Peptides, Reproduction

P-34: Evaluation of Sperm Abnormal Morphology in Male Rats Induced by Varicocele

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Background: Varicocele refers to the abnormal inflation of spermatic veins in the scrotum. Varicocele is a common cause of infertility in men who are clinically associated with sperm abnormal morphology. So, this study aimed to evaluation sperm abnormal morphology in male rats induced with varicocele experimentally.
Materials and Methods: In this study, 30 adult male Wistar rats were divided into three groups, I: varicocele-induced II: sham and III: control. After two months of varicocele induction, rats were sacrificed and epididymides were dissected. Then, Sperm abnormal morphology assessed by WHO protocol and eosin-nigrosine test. Differences within groups were compared by one-way analyses of variance (ANOVA) using a post hoc test (Tukey). Collected data were presented as mean ± standard error of mean (SEM) and $P<0.05$ was considered to be significant.
Results: The result of this study showed that mean Sperm abnormal morphology were significantly higher in varicocele induction group compared to control and sham groups ($P<0.01$). The deformity of tail and head of sperm in varicocele rats compared with sham and control rats showed a significant increase of ($P<0.05$) However, that sperm neck deformity in varicocele

groups increased but did not show significant difference.
Conclusion: In this study, by evaluating sperm parameters between groups, it was shown that the abnormal morphology of sperm in varicocele rats significantly increased, which was consistent with previous studies. Also, respectively highest sperm anomalies were the sperm tail and head, and then sperms that had been defeated from the neck was in the next rank.
Keywords: Varicocele, Sperm Parameters, Sperm Abnormal Morphology, Eosin - Necrosin Test

P-35: The Comparison of α -Tubulin Protein Expression in Normospermic and Oligoasthenoteratospermia Semen Samples

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Background: The centrosome is considered to be the microtubule organizing center and composed meshwork of proteins including α-tubulin. Sperm centrosome is located in the midpiece of the tail. It is believed that α-tubulin protein levels have been disturbed in oligoasthenoteratospermi. Hence this study was carried out to evaluate α-tubulin protein in normozoospermic and ligoasthenoteratospermic semen samples.
Materials and Methods: The semen samples were collected from 20 normozoospermic and 20 ligoasthenoteratospermic men who underwent seminal fluid evaluation at the Royan Institute. The conventional sperm parameters were assessed by computer-assisted sperm analyzer. The expression of α –tubulin protein were analyzed by Western Blotting. All values were expressed as mean ± SD.
Results: The levels of α -tubulin are markedly lower in oligoasthenoteratospermic samples (0.59 ± 0. 14) as compared to the normozoospermic samples (0.84 ± 0.32).
Conclusion: Lower α -tubulin protein expression in sperm of infertile males may be a possible cause for their reduced fertilization ability. Further studies on centrosomal protein are required to design rational approaches for the diagnosis and treatment of male infertility.
Keywords: Centrosome, α –Tubulin, Oligoasthenoteratospermia

P-36: Evaluation of Histological and Histochemical Alterations in Testis Tissue following Long-term Oral Administration of Aspartame in Mice

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Background: Aspartame is the most famous consuming artificial sweetener that widely used in foodstuffs. Many experimental studies have reported toxicity of long-term administration of aspartame in different organ tissues, whereas little evidence is available on adverse effects of long-term consumption of aspartame on reproductive system. The present study has been conducted in order to evaluation of the effects of aspartame on histological and histochemical parameters in mice.
Materials and Methods: The adult male mice were randomly divided into four groups of nine each. In three groups, aspartame was administered orally with the doses of 40, 80 and 160 mg/kg.BW respectively for 90 days by gavage. Also a control group was considered. 24 hours after the last treatment, tissue samples were taken and used for histological and histochemical evaluations (Masson's trichrome, Alkaline phosphatase, Oil red O, Sudan black B and Periodic acid Schiff stains).
Results: Microscopic analyses revealed that long-term administration of aspartame increased thickness of tunica albuginea, edema in subcapsular and interstitial connective tissues, and atrophied seminiferous tubules, arrested spermatogenesis, decreased Leydig cells/mm^2 of interstitial tissue, and causes hypertrophy and cytoplasmic granulation of Leydig cells. The carbohydrate ratio was reduced in first three layers of the germinal epithelium (GE) cytoplasm. The upper layers of the GE series were manifested with low rate of lipid accumulation in cytoplasm, while the cells which were located in first layers were revealed with higher amount of lipid foci. Also, histochemical changes were observed in testis tissue of 80 and 160 mg/kg groups.
Conclusion: The current study indicated that long-term oral administration of aspartame causes histological and histochemical adverse effects on the testicular tissue which potentially can lead to infertility.
Keywords: Aspartame, Histology, Histochemistry, Mice

P-37: PRKAR2B Expression at Different Times after HCG-Induced Ovulation in NMRI Mice

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Background: Oocyte maturation and resumption of meiotic occur by induction of hCG (Human chorionic gonadotropin) that hormone functions instead of the LH (Luteinizing Hormone). Diminishing The cAMP (cyclic adenosine monophosphate) level will control the phosphorylation/dephosphorylation of cAMP-PKA effective for the activation of maturation promoting factor (MPF) and the activation of MPF causes resumption of meiotic in immature oocytes. So the mechanisms cAMP-PKA regulate the meiotic status of oocytes. PRKAR2B (cAMP-dependent type 2 regulatory subunit beta) is expressed in ovarian follicles as well as other tissues such as white and brown adipose and brain. This gene produces PRKAR2B, one of the PKA members. Since hCG plays an important role in ovulation induction and according to the possible role of PRKAR2B gene in ovulation, the effect of hCG injection on the expression of PRKAR2B gene in MII oocytes was investigated. The real function of in the resumption of meiotic is unclear still.
Materials and Methods: In this study, female mice were randomly assigned to four treatment and control groups. In each group, all mice were under ovarian stimulation by 10 IU pregnant mare's serum gonadotropin (PMSG). After 48 hours, they were inducted by hCG 10 IU due to ovulation. Mice were sacri-

ficed by spinal dislocation at the hours 16, 20, 24 and 28 for the released oocytes and gene expression of PRKAR2B after ovulation induction. Results: The lower number of MII oocytes at hour 28 in comparison with those of other hours and PRKAR2B expression in the hour 24 group was significantly higher than those of other groups were seen while the expression was significantly lowest at hour 28.
Results: The lower number of MII oocytes at hour 28 in comparison with those of other hours and PRKAR2B expression in the hour 24 group was significantly higher than those of other groups were seen while the expression was significantly lowest at hour 28.
Conclusion: We conclude this relation between PRKAR2B and the rate of cell proliferation. In respect to the results of this study seems that the lower expression of PRKAR2B will result in the less yielded MII oocytes.
Keywords: HCG, Ovulation, PRKAR2B, Mouse

P-38: The Effect of Cult – Active Medium on Fertilization and Pregnancy Rates in Couples with Azoospermia Male Factor

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Background: To evaluate efficiency of cult active medium on fertilization and cleavage rates, embryo development, and pregnancy rate after intracytoplasmic sperm injection (ICSI) in obstructive azoospermia patients.
Materials and Methods: This study was done on 157 ICSI cycles (stimulated with standard long protocol). After oocyte collection, the oocytes were randomly divided into two groups: control and artificial oocyte activation (AOA). The injected oocytes in the control group were cultured in cleave. The remaining oocytes were chemically activated by exposure to 200 µl cult-active medium for 15 minutes. Around 16 to 18 hours after ICSI, fertilization was assessed. The percentage of cleavage and embryo quality were calculated 72 hours after ICSI. Pregnancy rate was determined by Biochemical experiment.
Results: There are significant differences in the fertilization and cleavage rates after Cult-active used ($P<0.05$). also the pregnancy rate significantly increased ($P<0.05$). embryo quality no difference between ICSI AOA and control groups ($P>0.05$)
Conclusion: The findings showed that Cult-active treatment may fertilization and cleavage rates, which in turn, affect the implantation and pregnancy rate.
Keywords: Cult-Active, Obstructive Azoospermia, Failed Fertilization, Oocyte Activation, ICSI

P-39: Human Placenta Decellularized Matrix as A Scaffold for Use in Proliferation of Spermatogonial Stem Cells

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Background: 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. Although various protocols have been developed to remove the cells from tissues with minimal degradation of ECM components, an optimal protocol is still needed to satisfy scientists. In this study, we examined various procedures for decellularization of placenta and optimized a reliable decellularization protocol.
Materials and Methods: Human placenta was obtained from mothers undergoing cesarean after obtaining informed consent. The tissues were treated with various contents of Triton X-100 and sodium dodecyl sulphate (SDS) for 15 or 30 minutes. The decellularized tissues were casted, freezed and freeze dried to fabricate a porous scaffold. The scaffolds were then cross-linked by glutaraldehyde gas. The removal of the cells from tissues was determined by HandE and 4′, 6-diamidino-2-phenylindole (DAPI) staining, and DNA content assay. Alcian blue, Masson's trichrome and Orcein staining was used to confirm that ECM remained intact after decellularization process. Morphology of porous scaffold was viewed under scanning electron microscopy (SEM). Cell viability and cell adhesion property of scaffold were determined by MTT and SEM.
Results: Histological analysis showed that the groups content %0.5 SDS and SDS+ Triton X-100 for 30 minutes were completely decellularized. Decellularization was further confirmed by DAPI staining and DNA content assay. Laminin, collaegen, elastin and glycosaminoglycans remained intact after decellularization process. MTT test showed cell viability changes for %0.5 SDS group. SDS+ Triton X-100 for 30 minutes have no significant difference with control group ($P>0.05$).
Conclusion: Our study proved a reliable and effective protocol for decellularization of placenta with minimal negative effects on ECM components. The decellularized placenta is suggested as a promising bio-scaffold tissue engineering applications.
Keywords: Decellularized Scaffold, Placenta, Extracellular matrix

P-40: Effects of Myo-Inositol on Sperm Parameters and DNA Integrity in Asthenozospermia after Cryopreservation

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Background: Human semen cryopreservation considered as indispensable part of assisted reproductive centers, andrology laboratories and sperm bank. Despite long history of semen cryopreservation, post thawing survival rate is still limited and fails to meet the ideal expectation. Accordingly, cryoprotective media are commonly supplemented with antioxidants and it has been shown to improve cryo-survival post thawing. Therefore, the aim of this of this study was to evaluate effect of myo-inositol during human semen cryopreservation.
Materials and Methods: A total of 20 semen samples were

collected from men with asthenozospermia parameters attended the Andrology Unit of the Qom Fertility and Infertility Center. Each semen sample was divided into two equal aliquots. The two identical aliquots of each semen sample were randomized into two groups (A and B). Group A was treated with cryo-protectant plus with (2 mg/mL) Myo-inositol solution, while group B was treated with cryo-protectant alone (control). The Sperm parameters after thawed analysis by WHO guidelines and DNA integrity were evaluated via acridin orange method.
Results: The results of this study showed that the sperm samples frozen with cryoprotectant (Myo-inositol) had a significantly higher proportion of sperm motility and sperm viability compared with those frozen without cryoprotectants ($P<0.01$). In addition, this study showed DNA integrity had significant difference between groups ($P<0.05$).
Conclusion: *In vitro* Myo-inositol supplementation of cryopreserved ejaculate sperm, from infertile men, resulted in a significant enhancement of post-thaw sperm quality. Such finding is interesting, and may have important implications on the future outcome of assisted reproductive techniques using cryopreserved sperm.
Keywords: Myo-Inositol, DNA Damage, Sperm Parameters, Cryopreservation

P-41: Evaluation of Dianabol Effects on Fertility Rate in Male Mice

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Background: Dianabol is an orally active, synthetic anabolic-androgenic steroid. Low doses of these drugs are used to treat diseases such as anemia, poor growth in children and to reduce the spread of HIV. The main subject of this study is determination of adverse effects of dianabol in fertility rate of adult male mice.
Materials and Methods: 18 adult male mice were divided into 3 groups of 6: Group 1: as control 0.2 ml distilled water were given daily through gavage. Group 2: received 5 mg/kg Dianabol daily by gavage. Group 3: were given 10 mg/kg of Dianabol daily by gavage. Treatment period was 6 weeks. At the end of the treatment, each mouse from each groups were coupled with three adult female mouse. Ten days after determining vaginal plaque, the animals were dissected and the number of embryos and corpora lutea were counted and fertility rate was examined.
Results: Fertility examinations revealed that 5 mg/kg BW and 10 mg/kg BW made significant decrease in fertility rate compared with control group. Also, fertility rate of group 3 was declined in comparison with group 2. Moreover, evaluation of Arrested embryos showed that Dianabol in group 2 and 3 caused significant increase the arrested embryo in comparison with control group.
Conclusion: Due to the results, it can be mentioned that dianabol considerably made dangerous effects on fertility specially increased arrested embryos. So, the athletes using dianabol must be careful in applying doses of this anabolic steroid.
Keywords: Dianabol, Fertility, Mice

P-42: Methandrostenolone High Doses Effects on Sperm Quality in Adult Mice

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Background: Methandrostenolone as Anabolic-androgenic steroid ranks among the drugs most widely abused with the goal of improving athletic ability, appearance, or muscle mass. This drug was developed as synthetic analog of testosterone and are administrated for treating refractory anemia, hereditary angioedema, breast cancer and starvation states. The doses of Methandrostenolone used by athletes is typically in excess of therapeutic doses. The aim of this study was to determine the effects of high doses of Methandrostenolone on sperm characteristics.
Materials and Methods: This study was carried on 18 adult male mouse. Animals were divided to 3 equal groups as follow: control, M10 and M20 groups that respectively received 0.2 ml distilled water, 10 mg/kgBW and 20 mg/kgBW Methandrostenolone daily through gavage for 42 days. After treatment period, animals were euthanized by cerebrospinal dislocation and dissected. Cauda epididymis was collected and put in PBS buffer, then cut into pieces. Finally, sperm motility, vitality and count were evaluated. one drop of sperm suspension was spilled on the slide and sperm motility was evaluated with microscope equipped to hotplate and the result was expressed as percentage of motile sperms. Also, eosin-nigrosin staining was used for assessment of sperm vitality and sperm count was performed with hemocytometer.
Results: Evaluation of sperm quality in all groups indicated that M10 and M20 groups caused significant decline in sperm quality in comparison with control group. Also M20 group showed significant decrease in sperm motility, vitality and count compared with M10 group.
Conclusion: Regarding to the considerable diminution of sperm quality in animals induced with high doses of Methandrostenolone, it may affect the fecundity of the users. So, we suggest specially to the athletes to eschew for applying high doses of Anabolic Steroid Methandrostenolone
Keywords: Methandrostenolone, Sperm Quality , Significance, Mice

P-43: Investigation of The Ovarian Histological Changes after Treatment with Ethephon in Mice

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Background: Ethephon including illegal chemicals uses in agricultural production, which is set to accelerate growth and achieve, improve the quality of the product. The most important side effects of ethephon on the body include cancer, liver disease, kidney disease, reproductive system dysfunction and infertility. The purpose of the study was to investigate the effect of ethephon on ovarian histological changes in mice.
Materials and Methods: In this study, 50 adult female mice

with an average weight of 30 g were used for the experiments. Animals were divided into five groups respectively: control (without receiving any substance), sham (receiving daily serum physiology orally), ethephon 1 (receiving daily 192 mg/kg orally), ethephon 2 (receiving daily 240 mg/kg orally), ethephon 3 (receiving daily 480 mg/kg). After 21 days, ovaries were separated for histological studies. Ovaries were embedded in paraffin and cut into sections 6mm thick. The sections were stained with Hematoxylin-Eosin (HE) and observed under an optical microscope.
Results: The results indicated that in the average dose (240 mg/kg) and high dose (480 mg/kg), the follicular atresia increased and hyperemia in different parts of the ovary especially in medial portion of the ovary was seen. In high doses, the most atresia was seen in the secondary follicles as a cavity formation.
Conclusion: It seems that oral administration of ethephon with effect on ovaries can have a deleterious effect on fertility and embryo development in mice.
Keywords: Ovarian Follicle, Atresia, Histological Changes, Ethephon, Mice

P-44: The Effect of Ethephon on Oxidative Stress Factors in Female Mice

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Background: Ethephon is a good and effective plant growth regulator that has the effects on promoting fruit ripening. The most important side effects of ethephon in the body include cancer, liver disease, kidney disease, impaired reproductive system performance, and infertility. This study was done to investigate the measurement of the oxidative stress system factors in serum samples of ethephon-receiving mice.
Materials and Methods: In this study, 50 adult female mice with an average weight of 30 g were used for the experiments. Animals were divided into five groups; control (without receiving any substance), sham (receiving daily serum physiology orally), ethephon 1 (receiving daily 192 mg/kg orally), ethephon 2 (receiving daily 240 mg/kg orally), ethephon 3 (receiving daily 480 mg/kg). Oxidative stress markers including malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured in serum samples. The data were analyzed using SPSS version 21 software and the results were expressed as mean ± SD.
Results: The results of the experiments showed that ethephon in all three doses reduced the TAC compared to the control group ($P<0.05$). Also the level of MDA in three doses of ethephon-treated mice increased compared to the normal control mice ($P<0.05$).
Conclusion: It can be concluded that an oral administration of ethephon with effect on the oxidative stress system can have a deleterious effect on fertility and embryo development in mice.
Keywords: Ethephon, Oxidative Stress, Fertility, Mice

P-45: Effect of Raffinose on The Cryopreserved Rooster Spermatozoa in Modified Beltsville Extender

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Background: Cryopreservation of sperm cells causes irreversible damage to the sperm. Factors such as the formation of ice crystals, the production of active oxygen species, temperature changes, lipid peroxidation, changes in membrane composition, and osmotic stress reduce the quality of sperm after thawing. Raffinose, composed of fructose, galactose, and glucose, is a sort of native oligosaccharide and is classified as a trisaccharide. Raffinose plays a cryoprotective role by interacting with membrane lipids and proteins and decreasing the risk of intracellular ice crystal formation, which causes cellular osmotic dehydration during cryopreservation. The present study was conducted to determine the effects of different levels of Raffinose on some post-thawed rooster semen quality parameters.
Materials and Methods: Semen samples were collected from eight sexually mature Ross 308 breeder roosters. After initial semen assessments, samples with adequate quality were mixed together and diluted with modified Beltsville extender without Raffinose (control) and supplemented with 50, 75, and 100 mM Raffinose. After thawing, sperm viability and motility were measured by Eosin-Nigrosine and Computer-Aided Sperm Analysis (CASA), respectively. The data were analyzed by the GLM procedure of SAS 9.1.
Results: Using Raffinose at 50 Mm, 75 mM, and 100 mM significantly increased sperm motility (60%, 50.25%, and 47.5%, respectively; $P< 0.05$), and viability (64%, 53.5%, and 51% respectively; $P< 0.05$) in compared with control. In terms of progressive motility, the extender supplemented with 50 mM and 75 mM Raffinose improved sperm progressive motility compared to the control group ($P< 0.05$). But using 100 mM Raffinose had no significant effect on sperm progressive motility.
Conclusion: The results of this study revealed that the addition of Raffinose to the diluent improves significantly the function of post-thawed rooster spermatozoa.
Keywords: Raffinose, Rooster, Semen, Cryopreservation

P-46: Histopathologic Evidence Associated with Impaired Folliculogenesis Induced by Butachlor in Rat Ovaries

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Background: Butachlor is one of the top members of the chloroacetanilide herbicide, extensively used for control of annual grasses and some broad-leaved weeds, particularly in rice crops. The studies of the possible toxic effects of butachlor have indicated that it is a suspected carcinogen and posing a potential threat to the agro-ecosystem and human health. The aim of this study was to evaluate the impact of butachlor on morphological and pathological changes of rat ovaries.
Materials and Methods: Twenty-eight female Wistar rats were housed under standard housing conditions (controlled 12:12 hour light/dark cycles and an ambient temperature of 22-24 °C). The rats were acclimatized for two weeks before the experiment. Vaginal smears were examined daily in order to select

normal estrous cycles in female rats. Female rats with regular estrous cycles were randomly divided into a control group or an experimental group to receive oral butachlor 72 mg/kg /day. Seven rats from each group were sacrificed after six weeks for blood collection from the dorsal aorta and removing the ovaries to pathological examination and measurement of ovarian follicles apoptosis. The same procedures were performed in the remaining rats form each group six weeks later. Ovaries tissue sections were prepared and stained with hematoxylin-eosin for morphometry by light microscopy. Serum levels of progesterone and estradiol were measured by ELISA.
Results: Serum levels of progesterone and estradiol showed no significant difference between the experimental and the control group (P>0.05). The number of primary and mature follicles statistically increased in the experimental group after 12 weeks of exposure. In addition, the primary follicles, as well as atretic follicles, had the highest mean number after 6 weeks of exposure in the experimental group compared to the control group (P<0.05).
Conclusion: This study demonstrated that butachlor negatively impact ovarian folliculogenesis and maybe ovulation. Under the influence of butachlor, the appearance of the ovary has changed to the cystic ovary. Butachlor likely influences metabolic systems that can exacerbate anovulation and the PCOS phenotype. Protective strategies and strong recommendations should be considered to decrease human exposure to this pesticide.
Keywords: Butachlor, Ovary, Rat

P-47: Cyclopiazonic Acid Lowered The Quality of Sperm Parameters and Fertility Rate in Mice

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Background: Mycotoxins are secondary active metabolites, which are produced by different species of fungi. Cyclopiosonic acid (CPA) is an indole tetrameric acid mycotoxin which is found in various nutrients (meat, milk and eggs). This study was aimed to investigate the potential effects of CPA at different dose levels on the sperm quality parameters and fertility rate in mice.
Materials and Methods: Forty adult male mice were randomly divided into 5 groups (n=8). The control group did not receive any treatment. In the control-sham, animals received daily 0.05% DMSO (as the CPA solvent) intraperitoneally (i.p.). In the third, fourth and fifth groups, animals received 0.3, 0.6 and 0.12 mg/kg, BW of CPA (i.p.), respectively for 28 days. At the end of treatment period, sperm quality parameters, sperm damage and the fertility rate were evaluated.
Results: CPA exposure for 28 days resulted in a significant reduction in sperm count, sperm motility, sperm survival and chromatin quality. At the same time, CPA elevated the percentage of sperms with damaged DNA. Reduced fertilization rate, two-cell embryos (%), blastocysts (%) and hatching embryos (%) were recorded in the CPA-received animals compared to the control group.
Conclusion: The detrimental effects of CPA as reproductive toxicity, may attribute to the alterations in sperm parameters and in vitro fertility rate reduction. Therefore, the presence of CPA in foodstuffs alone and/or along with other mycotoxins could have negative effects on the male fertility factors.
Keywords: Cyclopiazonic Acid, *In Vitro* Fertilization, Mice, Mycotoxin, Sperm

P-48: Effect of Pentoxifylline on Tumor Necrosis Factor and Reactive Oxygen Species Levels in Seminal Plasma in Idiopathic Male Infertility

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Background: Pentoxifylline is a methyl xanthine derivative that influences the sperm motion characteristics. It is commonly used in treatment of male-factor infertility, including asthenozoospermia. The aim of this study was to evaluate any possible effect of Pentoxifylline on sperm characteristics ،(TNF) tumor necrosis factor and (ROS) reactive oxygen species Levels in Seminal Plasma in a group of patients with asthenozoospermia.
Materials and Methods: 30 infertile men with asthenozoospermia were allocated to this study. A dosage of 400 mg pentoxifylline / twice daily for duration of 3 months was administered to each patient. Two semen samples (one before and one after the pentoxifylline therapy) were evaluated under blind condition. Semen parameters ,TNF and ROS Levels (by human TNF ELISA kit and Chemiluminescence assay- Agarwal respectively) in seminal plasma were analyzed (pre and post intervention) for each sample. Data was analyzed statistically using one-way ANOVA and Tukey's test .
Results: Pentoxifylline increased significantly the mean sperm count and normal morphology in the men with asthenozoospermia when compared to pre-administration. pentoxifylline was significantly effective on the fast progressive motility of sperm (P<0.01). Based on the results, mean concentration (± SD) of TNF in the seminal plasma was significantly increased in patients treated with pentoxifylline when compared to pre-administration. Moreover, ROS levels in seminal plasma of patients with asthenozoospermia decreased significantly compared to before administration of pentoxifylline.
Conclusion: Although our results demonstrate that oral therapy of pentoxifylline significantly increase the quality of sperm, especially motility from infertile men with asthenozoospermia, but more molecular studies are needed to elucidate the safety of pentoxifylline- administration.
Keywords: Pentoxifylline, Seminal Plasma, Asthenozoospermia, Infertility

P-49: Effect of Co-Administration of Pentoxifylline and Zinc on Total Antioxidant Capacity and Malondialdehyde Levels in Seminal Plasma in Idiopathic Male Infertility

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Background: Pentoxifylline is a methyl xanthine derivative that influences the sperm motion characteristics. It is commonly used in treatment of male-factor infertility, including asthenozoospermia and Zinc is known to influence several phases of sperm life, from germ cell development to spermiation. The aim of this study was to evaluate any possible effect of a combination of zinc and Pentoxifylline on sperm characteristics and Total antioxidant capacity and malondialdehyde levels in Seminal Plasma in a group of patients with asthenozoospermia.
Materials and Methods: Ninety men with asthenozoospermia in a doubleblind, randomized clinical trial were allocated for this study. They randomized to tree groups. Group I received Pentoxifylline / and zinc, group II, Pentoxifylline, group III, zinc . pentoxifylline and zinc twice daily for duration of 3 months was administered to each patient according to grouping . Finally, we compared pre and post intervention semen parameters of sperm, levels seminal total antioxidant capacity (TAC) and malondialdehyde levels (MDA) in all the specimen by ferric-reducing ability of plasma assay (FRAP) and tiobarbiotic acid (TBA) methods, respectively. Data was analyzed statistically using one-way ANOVA and Tukey's test .
Results: Pentoxifylline and zinc increased significantly the mean sperm motility, count and normal morphology in the Pentoxifylline and zinc treated groups when compared to pre-administration. The mentioned parameters increased significantly in the Pentoxifylline + Zinc group in comparison with Pentoxifylline and Zinc groups. Based on the results, mean concentration (± S.D) of TAC in the seminal plasma was significantly increased in the groups of pentoxifylline, zinc, and pentoxifylline + zinc compared to pre-administration. Moreover, MDA levels in seminal plasma of patients with asthenozoospermia decreased significantly compared to before administration in the groups of pentoxifylline, zinc, and pentoxifylline + zinc. also, TAC level in zinc + pentoxifylline group was significantly higher than zinc and pentoxifylline groups. In the event that, MDA was significantly reduced in the pentoxifylline + zinc group compared with the groups of pentoxifylline and zinc.
Conclusion: It is suggested that pentoxifylline be co-administered with antioxidants such as zinc, for the treatment of male infertility.
Keywords: Pentoxifylline, Zinc, Asthenozoospermia

P-50: Sperm miR-26a Tanscript Content in Normozoospermic Fertile and Infertile Men

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Background: Infertility is a reproductive disorder with multiple genetic and environmental causes which affects 15% of couples worldwide. With male factor responsible for half of the cases, approximately 7% of men encounter with infertility. and About 60-75% of male infertility cases are considered to be idiopathic. Currently, due to WHO guidelines, conventional semen parameters, including sperm concentration, motility, morphology, seminal volume and pH is being used for evaluation of male fertility potential which provide limited information and cannot distinguish fertile and infertile men on an individual basis. Consequently, clinicians are searching for more reliable seminal bio-markers to have more accurate prospective in the case of male infertility patients.
Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. Semen samples were collected from fertile (n=15) and infertile men (n=15) with normal sperm parameters according to the World Health Organization (WHO) guidelines. Real-time PCR was carried out to evaluate relative expression of sperm miR-26a transcript content in ejaculated sperm.
Results: Sperm concentration was significantly ($P<0.05$) lower in men with unexplained infertility compared with control group. Significant ($P<0.05$) lower content of miR-26a transcript in ejaculated sperm observed in infertile men than fertile control. Also, significant correlation ($r=0.369$, $P=0.045$) was seen between miR-26a transcript content and sperm morphology.
Conclusion: Our findings indicate that assessment of sperm expression profile reflect the quality of spermatozoa in etiology of unexplained male infertility and suggest that miR-26a sperm content transcript could be used as novel diagnostic marker for male infertility or potential treatment target.
Keywords: Unexplained Male Infertility, Normozoospermia, Sperm Transcriptome, miR-26a

P-51: Protective Effect of Heparin on Epididymal Sperm Quality in Ischaemic-Reperfusion Injury of The Rat Testicle

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Background: Testicular torsion is an urologic emergency that causes testicular damage and lead to reduced fertility or infertility. It appears that the main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release. Surgical detorsion is currently the only treatment and allows blood reperfusion. Even with successful surgical repair, loss of spermatogenesis and a significant increase in germ cell apoptosis may happen. Heparin is a naturally occurring anticoagulant produced by basophils and mast cells. The protective effects of heparin and its derivatives in IR injury have been evaluated in different systems including hepatic, gastrointestinal, urogenital systems and in pancreas, lungs and heart but no previous study has evaluated the effect of heparin in the prevention of IR injury in rat epididymal sperm quality.
Materials and Methods: Eighteen Wistar Albino male rats weighing 250–300 g were divided into three groups: sham (group S, n = 6); torsion/detorsion (group T/DT, n = 6), and heparin pretreatment (group Hep, n = 6). The left testes were rotated 720° clockwise for 2 h in the rats of the torsion– detorsion group(group T/DT). Rats in the treatment group underwent the same surgical procedure as the torsion–detorsion group but were also given heparin 800 IU/kg (Hep group) by an intraperitoneal route 30 min prior to detorsion. In sham group(group s),

the left testes were brought out through the incision and were placed back in the scrotum. After 2 h of reperfusion a left orchiectomy was performed and the sperm was collected from the epididymis and sperm characteristics such as sperm motility, sperm vitality, sperm count and sperm morphology were examined

Results: Heparin significantly enhanced sperm motility and normal morphology of sperms but sperm vitality and sperm count did not differ significantly in Hep group in comparison with T/DT group.

Conclusion: Our results suggest that heparin treatment has a protective role on IR-induced testicular injury.

Keywords: Testicular Torsion, Sperm, Heparin

P-52: Electrospun Nanofiber Assemblies for *In Vitro* Three-Dimensional Uterine Tissue Engineering

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Background: The purpose of using this three-dimensional construct is to make a sufficient thickness membrane as a part of the uterine endometrium to protect implantation of the zygote.

Materials and Methods: In this study, Polycaprolactone/Gelatin/Polydimethylsiloxne (PCL/G/PDMS) nanofibers with aligned and random fiber arrangements were used as models to study the growth of uterine endometrial cells. To imitate in vivo tissue structure, three-dimensional (3D) cell/micro-nanofiber constructs with cells embedded among micro-nanofiber layers were built via layer-by-layer assembly. This structure showed that aligned nanofibers in the 3D constructs continuously induced cell polarization and promoted and demonstrated the potential of 3D cell/nanofiber construct as a model for proliferation of uterine endometrial cells in a physiologically relevant environment. A layer-by-layer assembly approach was adopted to stack cell-seeded nanofiber mesh (aligned or random) into 3D multilayered constructs. Briefly, aligned and random nanofibers collected on an aluminum foil were evenly seeded with endometrial cells (106 cells/scaffold). After 24 hours, ten layers of cell-seeded nanofiber meshes were overlaid to each other. Following this approach, one type of 3D cell/nanofiber constructs with either aligned or random nanofibers were formed. To visualize cell morphology inside the 3D cell/nanofiber constructs, at days 7 and 14 after seeding, samples were fixed in 4% glutaraldehyde and cut into 5-µm thin sections. The sections were then stained with hematoxylin and eosin (HandE) to evaluate the cell distribution. SEM micrographs of the scaffolds were obtained before seeding of the cells.

Results: Histological results showed that the cells homogeneously distributed through the entire constructs, and formed an integrated connection with nanofibers. Cells cultured on 2D random nanofiber meshes exhibited a polygonal morphology. Similar morphology arrangements were also observed with the cells cultured in 3D "sandwich" constructs.

Conclusion: A layer-by-layer approach was taken to assemble cell-seeded nanofiber meshes into 3D constructs with precisely controlled organization of nanofibers for mimicking the isotropy (i.e., stacking random nanofiber layers) of uterus tissue. This 3D culture system allows us to understand nanofiber-induced cellular responses in a physiologically relevant environment and preparation for a proper engineered tissue.

Keywords: Tissue Engineering, Nanofiber, Human Endometrium Cells, Three-Dimensional

P-53: Ascorbic Acid Attenuates Cognitive Impairment in Ovariectomized Mice

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Background: Menopause is associated with increased memory impairment. Regarding the antioxidant property of ascorbic acid (AA), the present study was designed to evaluate the effects of AA on cognitive function and the level of brain-derived neurotrophic factor (BDNF) in the brain in ovariectomized (OVX) mice.

Materials and Methods: For this purpose, AA (100, 300 and 500 mg/kg/p.o.), was administrated daily in OVX mice for 30 days. Tactile learning was evaluated by novel object recognition task. Also, the levels of serum BDNF were measured.

Results: AA prevented from the deleterious effects of ovariectomy on learning memory (300 and 500 mg/ kg). The serum BDNF level was also increased in OVX animals treated with AA (100 and 500 mg/kg).

Conclusion: Collectively, the results of the present study suggest that AA might be an appropriate choice in loss or reduction of estradiol for the amelioration of cognitive impairment.

Keywords: Ovariectomy, Ascorbic Acid, Learning and Memory, BDNF

P-54: Evaluation of The Effect of Ketorolac on The Rate of Sperm Motility and Survival in Infertile Men with Oligo-Asthenospermia in Culture

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Background: Decreased semen quality is a major factor of male infertility. The primary mechanism of action responsible

for ketorolac's anti-inflammatory, antipyretic and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX) 1 and 2. It has been hypothesized that Ketrolac has impact on semen quality and quantity. In this work, we review and reveal the effect of ketorolac on sperm motility and vitality in infertile men suffering from oligoasthenoteratozoospermia *in vitro*.
Materials and Methods: In this study, 40 seminal fluids were obtained from 40 infertile males suffering from oligoasthenoteratozoospermia. After collection by floating method, the samples were randomly placed in ISM1 media in four different groups: the control group without any addition, group number one with 50ug/ml ketorolc, group number two with 100 ug/ml ketorolc, and group number three with 200 ug/ml ketorolc added to the media. Sperm motility and vitality were examined after 1, 24, 36, and 48 hours. The data was analyzed by SPSS software. A P value of less than 0.05 was considered statistically significant.
Results: The average percentage of motile sperms was measured in control group after the intervals of 1, 24, 36, and 48 hours and a significant reduction of motility (p\<0.001) was observed in higher time points compared to the first hour; there was no statistically significant difference (P=19.0) between the four groups. The average percentage of alive sperms was also measured in control group after the intervals of 1, 24, 36, and 48 hours and a significant reduction of vitality (P<0.001) was observed in higher time points compared to the first hour; there was a statistically significant higher decrease in the third group (P=0.01).
Conclusion: Ketrolac increases the sperm vitality in infertile males suffering from Oligo-Asthenospermia only in 200ug/ml dosage but has no effect on sperm motility.
Keywords: Ketorolac, Oligo-Asthenospermia, Sperm

P-55: Seminiferous Tubules Histoarchitecture Changes in Nandrolone Decanoate-Treated Male Rats

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Background: Nandrolone Decanoate as an anabolic steroid is used for physique- and muscle mass-enhancing purposes by athletes. The misuse of this drug has irreparable consequences such as male infertility.
Materials and Methods: Eighteen rats were randomly categorized into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of Nandrolone Decanoate weekly through intra-muscular injections, respectively. After 8 weeks, the testicles were harvested and the seminiferous tubules histoarchitecture was analyzed.
Results: The seminiferous tubules epithelium thickness in the Nandrolone Decanoate -receiving groups showed a significant decrease compared to the control group, while seminiferous tubules luminal diameter in the Nandrolone Decanoate -treated rats exhibited a significant increase compared to the control group.
Conclusion: High doses of Nandrolone Decanoate can have negative effects on male reproductive system.
Keywords: Nandrolone Decanoate, Reproductive System, Seminiferous Tubulest, Rat

P-56: Effect of Nandrolone Decanoate on Rat Sperm Characteristics

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Background: High doses of nandrolone decanoate can result in decreased testosterone synthesis leading to spermatogenic disorders and male infertility.
Materials and Methods: Eighteen adult male rats were randomly divided into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of nandrolone decanoate weekly through intra-muscular injections, respectively. After 8 weeks, spermatological parameters were evaluated in all experimental groups.
Results: Nandrolone decanoate administration significantly reduced sperm count, motility and viability compared to control group.
Conclusion: Misuse of nandrolone decanoate can result in spermatological damages causing reproductive disorders.
Keywords: Sperm, Nandrolone Decanoate, Male Rat, Fertility

P-57: Effect of Nandrolone Decanoate on Sertoli Cell and Testicular Tubular Differentiation Indices in Rats

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Background: Misuse of Nandrolone Decanoate, an anabolic steroid, can lead to serious side effects including male infertility.
Materials and Methods: Eighteen rats were randomly assigned into 3 groups; group 1 was served as a control and groups 2 and 3 received 3 and 6 mg/kg of Nandrolone Decanoate weekly through intra-muscular injections, respectively. After 8 weeks, the testicles were harvested and Sertoli cell index (SCI) and tubular differentiation index (TDI) were evaluated.
Results: The SCI and TDI indices showed significant reductions in Nandrolone Decanoate-treated groups compared to the control group.
Conclusion: The use of anabolic steroids such as Nandrolone Decanoate may cause testicular dysfunction.
Keywords: Nandrolone Decanoate, Seminiferous Tubulest, Sertoli Cell, Spermatogenesis, Rat

P-58: Optimizing of Mouse Autologous Serum Through Endogenous Granulocyte-Macrophage Colony-Stimulating Factor after Induced Inflammation by Casein and Evaluation of its Effect on Embryo Development

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Background: Research studies on reproductive mechanism of laboratory animals are essential for further advancement of assisted reproductive techniques (ART). One of these studies includes the assessment of the effect of types of sera in culture medium on development of pre-implantation embryos. This study evaluated for the first time the effect of different protein supplements (BSA), mouse serum and mouse serum which treated by casein; on development, ICM, TE and apoptotic cell number and expression of Oct4, Cdx2, Bax, Bcl2 and receptor of GM-CSF (Csf2ra) genes in blastocysts of NMRI strain mouse.
Materials and Methods: Mice were injected IP with 2 ml of an 0.2% (wt/vol) solution of casein in mouse tonicity phosphate-buffered saline (MTPBS) and at 3 hours after injection blood collected from heart and serum separated. Two pro-nucleuses stage embryos from in vivo were collected by oviduct flushing. The 2PN's were randomly divided into three groups. 1. culture medium supplemented with 4mg/ml bovine albumin serum (BSA), 2. culture medium supplemented with 10% mouse serum (M), and 3. culture medium supplemented with 10% mouse serum that treated by casein. Then embryos were cultured up to the blastocyst stage. In 4th group embryos were developed in-vivo in to blastocyst stage. The rate of blastocyst development, apoptotic rate and number of Inner cell mass and Trophectoderm of blastocysts were measured, also quantitative expression of Oct4, Cdx-2, Bax, Bcl-2 and GM-CSF receptor (Csf2ra) were performed in these groups, using RNA extraction and Real Time PCR.
Results: Serum GM-CSF levels were measured by ELISA kit 0.348 ηg/ml. The difference in the percentages of development to blastocyst stage in culture medium containing BSA, M, treated M serum by casein and in-vivo groups were not significant. There were no significant difference between ICM numbers but TE and total cells of blastocyst were significantly increased in in vitro groups. Embryos that cultured with BSA had significantly more apoptotic cells in comparison the other groups. Quantitative PCR analysis showed that the difference in the expression level of Oct4, Cdx2, Bax, Bcl2 and Csf2ra was not significant.
Conclusion: In this study developmental rate was similar to embryos that cultured in 2 ηg/ml recombinant GM-CSF even though serum GM-CSF levels didn't increase up to optimized dose (2 ηg/ml). Considering the fact that the GM-CSF is endogenous and appropriate developmental rate usage of this serum could be a possible alternative.
Keywords: Culture Medium, GM-CSF, Developmental Genes, Apoptosis Genes, Csf2ra

P-59: Seminiferous Tubules Histological Alterations in Oxymetholone-administered Male Rats

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Background: Oxymetholone, an anabolic steroid, is used extensively among athletes and can affect male fertility.
Materials and Methods: Twenty-one adult male rats were randomly divided into 3 groups; group 1 was served as a control and groups 2 and 3 received 5 and 10 mg/kg/PO of Oxymetholone daily, respectively. After 60 days, the testicles were harvested and the seminiferous tubules histology was examined.
Results: The seminiferous tubules epithelium thickness in the Oxymetholone-administered groups showed a significant decline compared to the control group, whereas seminiferous tubules luminal diameter in the Oxymetholone-received rats exhibited a significant increase in comparison with control group.
Conclusion: High doses of Oxymetholone may have negative impacts on male reproductive system.
Keywords: Oxymetholone, Male Reproductive System, Seminiferous Tubule, Rat, Histology

P-60: Effect of Oxymetholone on Spermatogenic Indices in Rats

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Background: Oxymetholone as an anabolic steroid used extensively among athletes for muscle mass enhancing can lead to testicular malfunctions.
Materials and Methods: Twenty-one adult male rats were randomly categorized into 3 groups; group 1 was served as a control and groups 2 and 3 received 5 and 10 mg/kg/PO of Oxymetholone daily, respectively. After 60 days, the testicles were harvested and spermiation index (SPI) and tubular differentiation index (TDI) were recorded.
Results: The SPI and TDI indices exhibited significant declines in Oxymetholone-administered groups in comparison with control group.
Conclusion: Anabolic steroids abuse can lead to severe spermatogenesis impairment.
Keywords: Seminiferous Tubules, Oxymetholone, Spermatogenesis, Rat

P-61: The Effects of Myo-Inositol on Mitochondrial Function and Oxidative Stress Condition in MII Oocytes of PCOS Model Mice

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Background: Polycystic ovary syndrome (PCOS) is associated with hyperandrogenism, polycystic ovaries, infertility due to

ovarian dysfunction and menstrual irregularity and ultimately oocytes whit poor quality. The administration of myo-inositol (MYO) was associated with a decreased of serum testosterone, increase insulin sensitivity and improves the oocytes' quality. Inofolic is a dietary supplement for women with PCOS that contains myo-inositol (2 g) and folic acid (200 mcg).
Materials and Methods: Female NMRI mice were treated with a vehicle control or DHEA (6 mg /100 g body weight) or DHEA plus inofolic (0.37 mg /g body weight) for 20 consecutive days. After 20 days, Mature oocysts (MII) were retrieved from isolated ovaries . For inner mitochondrial membrane potential (MMP) or reactive oxygen species (ROS) or reduced glutathione (GSH) staining, denuded MII oocytes were incubated in PBS–PVA containing JC-1, H2DCFDA and Cell Tracker Blue fluorochromes respectively. After incubation, oocytes were washed and fluorescence was observed using a fluorescence microscope and fluorescence intensity was measured with Image J software.
Results: Significantly GSH and MMP were lower and ROS was higher in DHEA-treated oocytes compared with vehicle-treated. In DHEA+inofolic group, GLUT and MMP were significantly increased in contrast to the DHEA-treated and, on the contrary ROS fell.
Conclusion: It is likely that myo-inositol plays an important role in reducing ROS , increasing the antioxidant capacity, reducing cellular apoptosis and follow by increasing MMP and ultimately improving mitochondrial function in mice MII oocytes of PCOS model.
Keywords: Myoinositol, Polycistic Ovary Syndrome, Reactive Oxygen Species, Reduced Glutathione, Inner Mitochondrial Membrane Potential

P-62: Benzoic Acid-loaded Solid Lipid Nanoparticles Enhances Endometrial Receptivity through Upregulation of LIF and Integrin αVβ3

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Background: Embryo implantation is the crucial step for a successful pregnancy. Diverse factors, including adhesion molecules, growth factors, and cytokines are important for embryo implantation through improving endometrial receptivity. Benzoic acid (BA) is an aromatic carboxylic acid, whose positive effects on endometrial receptivity have been demonstrated, but the poor water solubility and low bioavailability have limited its therapeutic potential. Therefore, employing a nanoparticulate delivery system may enhance BA bioavailability. The present study proposed to develop solid lipid nanoparticles (SLNs) as a delivery system for improving BA effects on endometrial receptivity.
Materials and Methods: BA-loaded SLNs was prepared by hot homogenization technique and the nanoparticles characteristic include size, encapsulation efficiency and morphological behavior was determined by dynamic light scattering technic, ultrafiltration method and Scanning electron microscopy (SEM), respectively. Cytotoxicity of BA and prepared SLNs on endometrial cell line was evaluated by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Finally endometrial cells were treated with BA and BA-loaded SLNs for 48 h and expression of receptivity related genes evaluated by quantitative reverse transcription polymerase chain reaction (qRT-PCR).
Results: The nanoparticles with appropriate characteristics (particle size of 90 nm and Encapsulation Efficiency of 81%) were prepared. BA-loaded SLNs displayed a good stability for 4 weeks of storage at 4-8°C. No apparent cytotoxicity for SLNs and BA was considered which indicating the biocompatibility of the nanocarriers. Expression experiments revealed that BA and BA-loaded SLN upregulate expression of leukemia inhibitory factor (LIF) and Integrin αVβ3. Results also demonstrated that upregulation of LIF and Integrin αVβ3 will intensify when BA is loaded into SLN suggesting capability of SLNs in the more precise delivery of BA into cells than free BA.
Conclusion: The results strengthen our hope that loading BA into SLNs could possibly overcome the therapeutic limitations of BA and make it more effective in enhancing endometrial receptivity.
Keywords: Benzoic Acid, Receptivity, Endometrium, SLN

P-63: Protective Effect of Misoprostol on Ibuprofen-Induced Alteration of Sperm Parameters in Adult Male Mice

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Background: Ibuprofen, a propionic acid derivate is a non-steroidal anti-inflammatory drug (NSAID). In recent years several studies have been published on animal and human clinical experiments with NSAID, pointing to the deleterious effect of prostaglandins (PGs) on spermatogenesis. Misoprostol is a synthetic prostaglandin E1 methyl analogue indicated for the prevention of gastric ulcers induced by NSAIDs. The aim of the present study was to determine the protective effect of misoprostol on ibuprofen-induced alteration of sperm quality and quantity parameters in adult male mice.
Materials and Methods: In this study, 80 adult male mice were divided into 8 groups including: control, ibuprofen treated group which received 6 mg/Kg/day ibuprofen, three misoprostol treated groups which received 1, 10 and 100 µg/Kg/day misoprostol and three ibuprofen+misoprostol treated groups which received 6 g/Kg/day ibuprofen with 1, 10 and 100 µg/Kg/day misoprostol. All treatments were carried out for 40 consecutive days by oral gavage. At the end of experiment, animals were euthanized and their left epididymis was removed and dissected in Ham's F10 and incubated at 37°C. Total count, motility, viability and morphology of sperm as well as tratozoospermia (TZI) and sperm DNA fragmentation index (SDFI) were assessed according to the WHO standard methods. Data were statistically analyzed by SPSS using one-way ANOVA test and Tukey's post-hoc.
Results: Results showed that ibuprofen reduced total count, motility and viability of epididymal sperm and increased abnormalities, TZI and SDFI significantly compared to control. It was also indicated that high dose of misoprostol could increase motility and viability of sperm and decreased TZI and SDFI significantly compared to control group. Our results revealed

that all three dose of misoprostol could significantly increase total count, motility and viability of sperm and reduce sperm with abnormal morphology, TZI and SDFI compared to ibuprofen treated group in a dose dependent manner.
Conclusion: Based on our results it can be concluded that administration of misoprostol can improve quality and quantity indices of sperm and can be considered as a suitable protective strategy for improvement of male infertility or subfertility due to NSAIDs administration.
Keywords: Misoprostol, Ibuprofen, NSAID, Sperm, Male Infertility

P-64: The Association of Mass and Individual Sperm Motility with Abnormal Sperm Morphology

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Background: Sperm motility is believed to be one of the most important parameters in evaluating the fertilizing ability of ejaculated sperm, and fertilization rates of human oocytes in vitro have been shown to correlate closely with sperm motility. Both sperm motility parameters and percent normal morphology are significant factors in predicting fertilization and pregnancy rates. The purpose of this study was to determine the relationship between the sperm motility and abnormal sperm morphology.
Materials and Methods: Testis samples were obtained from 45 rams. The viability and abnormal morphology parameters of the cauda epididymal sperm were assessed by means of the Eosin-Nigrosin stain method. The viability and sperm abnormalities were assessed by counting 300 sperm cells in a microscope at 1000× magnification, using immersion oil. The cauda epididymal sperm motility was assessed in a light microscopy at 400× magnification at 37ºC. A computer-assisted sperm motility analysis (CASA) was used to analyse sperm motility.
Results: According to the statistical analysis, significant correlations were found between coiled principal piece and end piece of tail and mass motility ($P<0.01$) and mass motility and individual motility ($P<0.01$). Also, significant positive correlation existed between live sperm and detached head ($P<0.01$), coiled principal piece and end piece of tail and coiled midpiece of tail ($P<0.01$), slender head and macro cephalic ($P<0.01$), pyriform head and twin head ($P<0.05$).
Conclusion: Observation of individual and mass motility and estimation of the percentage of progressively motile sperm will provide information about sperm membrane integrity, as well as the morphologic integrity of spermatozoa. In conclusion, in this study mass motility correlated significantly with coiled principal piece and end piece of tail and individual motility.
Keywords: Sperm Motility, Abnormal Sperm, Morphology, CASA, Epididymal Sperm

P-65: LIF Can Increase The Expression of The αvβ3 Integrin in Cultured Mouse Blastocysts

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Background: Leukemia inhibitory factor (LIF) and integrins play an essential role in the interaction of trophoblast with endometrial cells in the implantation process. It has been determined that LIF regulates the expression of endometrial αvβ3 Integrin, but its role in αvβ3 expression on trophoblast cells is still unclear. Blockage of LIF has a negative effect on the implantation of mouse embryos. The aim of this study was to evaluate the effect of LIF on development of 8-cell mouse embryos into blastocyst stage and also expression of αvβ3 integrin, Bax, and Bcl-2 genes in resulted blastocysts.
Materials and Methods: The 8-cell mouse embryos were obtained from superovulated NMRI mice. The collected embryos were divided into four groups and cultured for 72 hours as follows: group 1. LIF free simple embryo culture; group 2. simple embryo culture with 1000 U/ml LIF; group 3. embryo co-culture with Ishikawa cells without LIF; group 4. embryo co-culture with Ishikawa cells with 1000 U/ml LIF. Embryo development was recorded every day and Cell counting was performed on the obtained blastocysts. As well expression of αvβ3, Bax, and Bcl-2 genes were evaluated in blastocysts of each group using real-time PCR.
Results: The results showed that the percentage of embryos that reached the blastocyst stage, number of cells, embryo survival rates and expression of αvβ3 integrin gene were significantly higher in the co-culture groups and simple culture with LIF group than LIF free simple culture group.
Conclusion: According to our findings, it seems that LIF can improve the growth, number of cells and survival of the embryos and Increases the gene expression of αvβ3 integrin in single blastocysts which has an important role in the success of implantation. The current results may provide a new approach to increase the implantation rate in infertile women.
Keywords: LIF, Embryo Implantation, αvβ3 Integrin, Blastocyst, Trophoblast

P-66: Association between Seminal Prolactin and Sperm HSP90 Transcript Content

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Background: Heat shock proteins (HSPs) are a group of proteins which have ability to protect cells against apoptosis and oxidative stress. To investigate role of seminal prolactin on men

fertilizing capacity, this study evaluated correlation between seminal prolactin (PRL) levels with mRNA content of HSP90 in ejaculated sperm.
Materials and Methods: Present study was approved by the institutional review board of Biology Department, Shahid Chamran University of Ahvaz. Sperm parameters were analyzed according to the World Health Organization (WHO) guidelines in men attending an infertility clinic and categorized to normozoospermic and asthnozoospermic groups. Seminal PRL levels assessed via radioimmunoassay method. Real-time PCR was carried out to evaluate mRNA content of HSP90 in ejaculated sperm.
Results: Significant (P<0.05) higher levels of seminal PRL was seen in normozospermic than asthnozoospermic men. Sperm content of HSP90 transcript was significantly (P<0.05) higher in asthnozoospermic than normozospermic men. Significant (r=-578, P<0.01) correlation was seen between levels of seminal PRL and sperm mRNA content of HSP90.
Conclusion: These findings show the significance of seminal PRL in relation to sperm HSP90 mRNA content and show that seminal PRL level has important clinical significance and could be considered as a diagnostic tool in prediction of male infertility.
Keywords: Semen Quality, Oxidative Stress, Asthenozoospermia, Prolactin, HSP90

P-67: Improvement of *In Vitro* Sperm Movement Characteristics in Normozoospermic and Asthenozoospermic Men by Repaglinide

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Background: Human sperm activity must be precisely regulated to achieve natural fertilization. The human sperm motility and hyperactivation induce by the different factors such as intracellular calcium concentration. Repaglinide is one of the current antidiabetic drugs that decrease blood glucose level by inducing the release of insulin in pancreatic islets. Mechanism action of repaglinide is blocked of ATP-sensitive potassium channels and depolarization of b- cell membrane, opening the voltage-gated calcium channels and then increasing in intracellular calcium. In this study; we examined the effect of repaglinide on in vitro induction of the human sperm motility and hyperactivated motility.
Materials and Methods: Semen samples were collected from two groups of normozoospermic donors and asthenozoospermic patients that were washed free of seminal plasma and then treated with medium alone (control) and were treated with 100 nM and 1µM concentration of repaglinide. After 1 hour of incubation for all treatments, the percentage of sperm motility and sperm hyperactivation were assessed.
Results: Results showed that repaglinide at concentration of 100 nM and 1µM caused significantly improvement of in vitro induction of sperm motility and hyperactivated motility in both groups, but in normozoospermic 1µM concentration of repaglinde has obviously effect on progressive motility and in asthenozoospermic group, highest hyperactivated motility rate was seen in 100 nM concentration of repaglinide in comparison to 1µM concentration and control ($p < 0.05$).
Conclusion: Our results suggest that repaglinide can improve sperm motility and hyperactivity in normozoospermic and asthenozoospermic men.
Keywords: Repaglinide, Motility, Sperm, Hyperactivation

P-68: The Effect of Clomiphene Citrate on Oocyte Quality via Expression of Growth Differentiation Factor-9 in Mice with Polycystic Ovarian Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women which influences 5 to 26 percent of them during reproductive age. Clinical features consists of menstrual disorders, hirsutism, acne, alopecia and irregular ovulation. Clomiphene citrate is an anti-estrogen combination which is used to increase LH and FSH levels to develop ovulation and improve fertility, can induce ovulation in approximately 80 percent of women with ovulation disorders. Various studies have demonstrated that growth differentiation factor-9 (GDF-9) can be considered as a marker in determining the quality of oocyte. The aim of this study was to determine the expression of GDF-9 gene in the oocytes of PCOS mice treated with clomiphene citrate.
Materials and Methods: In this experimental study, 18 adult female NMRI mice (25-30 g) were studied and divided randomly into 3 groups as follows: i. healthy control, ii. PCOS, and iii. treatment groups. The PCOS mice were induced by single intramuscular injection of estradiol valerate. Animals in treatment group were treated with clomiphene citrate for 10 days subsequent the induction of PCOS. Total RNA was extracted from oocytes and the expression of GDF-9 was determined using real time PCR. The level of blood sexual hormones were analyzed using ELISA method.
Results: Our results showed that GDF-9 expression level altered in the group treated with clomiphene citrate compared to the PCOS group which difference was significant at p value of 0.05. FSH and LH hormones level was also improved against the PCOS group.
Conclusion: The Clomiphene citrate treatment can improve the quality of oocytes in PCOS mice. This result could be under consideration of infertility treatment methods.
Keywords: Gene Expression, Clomiphene Citrate, Oocyte, Polycystic Ovarian Syndrome

P-69: The Effect of Pentoxiphyline on Sperm Parameters and Biochemical Factors in Mouse Treated with Dexamethasone

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Background: Infertility in men's crowd is increasing and use a series of medications such as dexamethasone is one of the main reasons for infertility. Pentoxiphyline as a potent antioxidant is able to reduce oxidative stress and the harmful effects of dexamethasone. The aim of this study was to evaluate the preventing effect of Pentoxiphyline on sperm parameters and biochemical

factors in mouse treated with dexamethasone.
Materials and Methods: This study 24 Adult male NMRI mice (35 ± 2 g) were divide randomly into 4 groups (n=6): control, Dexamethasone (7 mg/kg/day), pentoxiphyline (200 mg/kg/day), Dexamethasone + pentoxyphiline. After 7 days of treatment, the left caudal epididymis was cut in the Ham's F10 and the released spermatozoa were used to analyze sperm parameters, biochemical factors and daily sperm production were also measured. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at P<0.05.
Results: Motility, number, viability, sperm tail length and daily sperm production (P<0.001), Serum testosterone level (P<0.001), TAC (P<0.001) while in the volume of interstitial tissue and MDA level significantly increased in the Dexamethasone group when compared to the control. Co-administration of Dexamethasone and pentoxiphyline didn't significantly compared to control group on the above parameters Co-administration of Dexamethasone and pentoxiphyline didn't significantly compared to control group on the above parameters (P>0.05).
Conclusion: This study showed that simultaneous treatment of pentoxiphyline and dexamethasone can prevent the adverse effects of on sperm parameters, biochemical factors and daily sperm production.
Keywords: Pentoxiphyline, Sperm Parameters, Dexamethasone, Biochemical Factors, Mice

P-70: Effect of Vitamin E on Sperm Parameters and Expression of ODF Gene in Infected Mice with Chlamydia Trachomatis

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Background: Some bacteria example Chlamydia Trachomatis is the most prevalent bacterial cause of sexually transmitted infections in the world and can result in severe genital disease. Over 90 million chlamydial infections are detected annually worldwide and various studies have estimated that there are four to five million new cases of chlamydial infection each year in the USA. The aim of this study was to investigate the harmful effects of Effect of vitamin E on sperm parameters and Expression of ODF gene in infected mice with Chlamydia Trachomatis.
Materials and Methods: 24 Adult rats were divided into four groups: control, infected with C. Trachomatis, infected with C. Trachomatis + Vitamin E, Vitamin E (100 mg/kg/day). treatments were performed till 4weeks. Left caudal epididymis was cut in Ham's F10. Released spermatozoa were used to analyze number, motility and viability of the sperm also the expression of ODF gen was analyzed by real-time PCR.
Results: Our results showed treatment of animals with C. Trachomatis significantly decreased of Motility, number, viability, sperm tail length and daily sperm production (P<0.001) and ODF gene expression compared to the control group. While C. Trachomatis +Vitamin E group showed a highly significant increase in sperm parameters and ODF gene expression.
Conclusion: Vitamin E could compensate the adverse effects of C. Trachomatis on sperm parameters in adult mices.
Keywords: Adult Mice, Sperm Parameters, Vitamin E, C. Trachomatis

P-71: Effects of Biolozax-H on The Testis Structure and Pituitary- Gonadal Hormones in Adult Rat following Treatment with Clinical Dose

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Background: The activity of the renin-angiotensin system has an effect on oxidative stress in many tissues, including the testes. Therefore, in this study, the biolozax-H drug, which has an effect on the spermatogenesis activity and Pituitary- Gonadal Hormones in male rats, is further investigated.
Materials and Methods: Male adult rat (200-250 g) were divided into two groups of sixteen each. Control and biolozax-H treated group were administered 20 mg/kg biolozax-H for 45 days, respectively. The animals were sacrificed 45 days after starting treatment. The histological change on germinal epithelium, and Level of hormones was measured by Radioi Immuno Assay (RIA) method. Then, tissues were fixed in Buin's fixative. Sections were cut into 5 µm thicknesses and stained with Hematoxylin and Eosin (Hand E). The achieved results were analyzed in SPSS software using t test and ANOVA.
Results: Count of spermatogenic, Sertoli and leydig cells significantly decreased in the experimental group in comparison with the control and Control groups (P<0.05).also In the experimental group, titer of FSH, LH and testosterone hormone decreased significantly (P<0.05) in comparison with Control.
Conclusion: Regarding physiological role of Sertoli cells during spermatogenesis, reduction of FSH hormone may lead to negative effects on the sperm production and reproductive potential of male Rat.
Keywords: Biolozax-H, Sperm Viability, Testis, FSH, LH

P-72: Effect of Metronidazole in Sperm Progressive Motility, DNA Fragmentation and Sperm Quality Parameters in Adult Rat

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Background: Infertility is one of the major problems in the world,sperm progressive motility has been reported to be one of the key factors. Influencing in vitro and in vivo fertilization rates.there has been an increase in the literature of studies investigated with other semen parameters,however, few reports focused on the relationship between sperm DNA fragmentation and progressive sperm motility.
Materials and Methods: Male adult rat (200-250 g) were divided into two groups of sixteen each. Control and metronidazole treated group were administered 300 mg/kg metronidazole for 90 days, respectively. The animals were sacrificed 90 days after starting treatment. The histological change on germinal epithelium, sperm quality parameters (count and normal morphology), viability and DNA fragmentation on sperm were analyzed by light microscopy, computer-aided sperm analyzer (CASA).
Results: Metronidazole administration significantly decreased parameters of sperm (count and normal morphology) and increased germinal epithelium destruction. The head, mid piece and tail abnormalities of treated group were increased signifi-

cantly versus control. Higher levels of TUNEL positive cells that were found in treated groups demonstrated the increasing of DNA fragmentation in sperms following metronidazole treatment.
Conclusion: Overall ,our data suggest that sperm DNA damage is strongly associated with percentage of motility.
Keywords: Metronidazole, Sperm Motility, Epididymal Sperm, DNA Fragmantation

P-73: Granulosa Cells Morphological Changes in Infertile Women with PCOS Compared to Healthy Subjects Undergoing IVF

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Background: During follicular development, intraovarian paracrine signals coordinate granulosa cells (GCs) proliferation, theca cell differentiation, and oocyte maturation. In women with polycystic ovary syndrome (PCOS), ovarian hyperandrogenism and altered intrafollicular paracrine signaling perturb follicular survival, growth, and selection, causing accumulation of small antral follicles within the periphery of the ovary. Morphological Changes of (GCs) have not been thoroughly investigated during this syndrome.
Materials and Methods: The study was performed on ovarian GCs of 220 women who referred for IVF-ET. The PCOS group consisted of 120 PCOS patients, and 100 women with healthy ovaries were considered as the normal group. The syndrome was diagnosed by a gynecologist according to Rotterdam criteria (Rotterdam ESHRE/ASRM, 2004). After washing and trypsinization with 0.25% trypsin/EDTA solution, isolated GCs were resuspended in DMEM/F12 culture medium, and counted by a hemocytometer. Trypan blue 0.4% was used to determine the viable and total cell counts. GCs were seeded in 6-well plates (2×10^6 cells/well) and cultivated in 3 mL DMEM/F12 supplemented with 5% Fetal Bovine Serum , 1% L-Glutamine, and 1% Penicillin/ Streptomycin for 48 hours. In order to evaluate the purity, morphology, and growth rate of GCs, cell culture plates photography was done, and images were analyzed by ImageJ software.
Results: Typical morphology of normal GCs (N-GCs) was spherical. However, spherical and non-spherical cells were observed in both groups, but the ratio of spherical to non-spherical PCO-GCs was significantly ($P< 0.0001$) lower than that N-GCs (0.36 ± 0.02 vs. 7.34 ± 0.38). Photographs analysis showed that the mean value of the diameter of spherical PCO-GCs were significantly ($P<0.0001$) smaller than N-GCs (6.022 ± 0.054 vs. 10.21 ± 0.058 μm). Trypan blue exclusion test showed significant difference in viable GCs percent between PCOS patients and normal women ($72.93 \pm 2.12\%$ vs. $83.36 \pm 2.42\%$).
Conclusion: The current study findings suggest that metabolic changes in GCs of women with PCOS undergoing IVF-ET correlate with significant morphological abnormalities and decreased viability. Due to bidirectional communication between GCs with oocyte, the quality of the oocyte, consequently, the quality of the embryo are adversely affected.
Keywords: Morphology, Viability, Granulosa Cells, PCOS

P-74: Study The Protective Effect of Kombucha on Reducing The Adverse Effects of Silver Nanoparticles on the Indexes Oxidative Stress (FRAP, MDA) and Serum Testosterone Hormones NMRI Mice

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Background: Today, nanotechnology is one of the most relevant topics in the world of science. Among nanoparticles, silver nanoparticles are widely used in modern technology, especially in medicine. Due to their small size, these particles pass through the cell membrans and blood testis barrier and even lead to oxidative stress in the male reproductive system. Kombucha is a traditional fermentation drink with a strong antioxidant properties that evaluates the anti-oxidant activity of the Kombucha by performing DPPH and ABTS tests. The aim of this study was to investigate the protective effect of as a potent Kombucha antioxidant against the effects silver nanoparticle on blood serum testestron and the level of lipid peroxidation in mice.
Materials and Methods: Adult NMRI mice were randomly divided into 4 groups (n=6), control, Silver nanoparticles (500 mg/kg/day), Kombucha extract (9 ml/kg/day) and Silver nanoparticles + Kombucha extract, and treated for 35 days. Blood Samples Were teken form the heart.Serum Samples Were collected and biochemical evaluations used. Cambacha was prepared industrially, then the amount of polyphenols and DPPH and ABTS radicals and its carbohydrates, as well as glucuronic acid (by HPLC), were measured. The results were analyzed by one-way ANOVA and Tukey's test and the means were considered significantly different at $P<0.05$
Results: There was a significant decrease in testosterone levels in the serum of treated rats with silver nanoparticles ($p <0.05$) and there was a significant increase in antioxidant capacity. Moreover, malondialdehyde level in this group was significantly higher than that in the control group. ($P <0.05$). The above parameters in the nanoparticle group + kambuca were largely compensated for the silver nanoparticle group.
Conclusion: The Kombucha extract was able to protect against the seminal vesicle damage caused by silver nanoparticles by reducing the oxidative stress.
Keywords: Silver Nanoparticles, Kombucha, Testosterone, Oxidative Stress

P-75: Mancozeb Induced Cell Death in Sertoli-Germ Cells Co-Culture

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Background: Exposure to many substances such as pesticides have detrimental effects on health and are considered to contrib-

ute substantially to most diseases. They mediate some of toxic effects by regulation/induction of apoptosis, which ultimately lead to impaired fertility. Mancozeb (MZB) is a fungicide routinely used to protect field crops against fungal diseases. It has been shown to produce hepatotoxicity, neural disorders, thyroid glands and reproductive dysfunctions. We investigated whether MZB induces cell death in Sertoli-germ cells.
Materials and Methods: Experiments were carried out in Sertoli- germ cells from mice. Testes were decapsulated and cells were obtained by mechanical and enzymatic digestion. Sertoli-germ cells were seeded in complete DMEM/F12 supplemented with FBS. Cells were treated with the appropriate concentrations of MZB (1.5, 2.5 µM) for 3 hours For assaying apoptosis, cells harvested, washed, and stained with Annexin V and PI. They were incubated at room temperature for 15 minutes in dark. Cell fluorescence was acquired by flow cytometer system
Results: Flow cytometric analysis showed that MZB caused apoptotic cell death after treatment with 1.5, 2.5 MZB (significant difference: P< 0.001, compared with control).
Conclusion: In summary, result of the current study suggests that MZB can induce cell death which may eventually affect the production of sperms.
Keywords: Mancozeb, Sertoli-Germ Cells, Apoptosis

P-76: Regulatory Effect of Granulocyte-Colony Stimulating Factor on Natriuretic Peptide Precursor Type C During Pre-Ovulatory Period and Successful Implantation of Embryos from Mating in NMRI Mice

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Background: Natriuretic peptide precursor type C (NPPC) is expressed by ovarian follicles. This factor participating in the maintenance of oocyte meiotic arrest at the diplotene stage of prophase I for a long time. G-CSF (Granulocyte-colony stimulating factor) is one of the hematopoietic growth factors and produce in developing follicles, fetal and reproductive tissues. G-CSF has recently been shown to be involved in oocyte maturation and ovulation. In this study we determined whether or not G-CSF plays a role in the regulation of NPPC in resumption of oocyte meiosis and also its effect on fetuses.
Materials and Methods: Immature female NMRI mice were randomly assigned to control and treatment groups were injected i.p. with 10 IU of PMSG (Pregnant Mare's Serum Gonadotropin) to stimulate follicle development. Treatment group were received G-CSF (50 µg / kg i.p.), at the time of PMSG administration, while the control group had the same volume of normal saline instead of G-CSF at the same time. 48 hours post-PMSG administration Pre-ovulatory follicles were collected for quantitative real-time PCR analyses. Also, the above agents were injected into mature mice and 48 hours after injecting PMSG, in order to stimulate ovulation in mature mice injected i.p. with 10 IU hCG (Human chorionic gonadotropin). On day 16 post coitus, the mature female mice of both groups were sacrificed for withdrawing their fetuses to determine their specification.
Results: The expression levels of NPPC have a significant decrease (P <0.05) in the treatment group compared to the control group. In other hand the weight of fetus in treatment group (0.771 ± 0.028) were significantly more than that of control group (0.667 ± 0.026) (P<0.05). Also no significant changes were found between follicle count, fetal number, CRL (Crown rump length), implantation and absorbed embryo.
Conclusion: GCSF by reducing the expression of the NPPC gene may increase fetal growth.
Keywords: G-CSF, NPPC, Gene Expression, Fetus, Mice

P-77: Effect of Polyvinyl Alcohol on The Motility, Morphology and Viability of Human Sperm after Thawing

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Background: Sperm cryopreservation has been used widely since the 1970s to store sperm in patients undergoing cancer therapy and play a vital role in treating couples with infertility. However, Cryopreservation lead to induce different in cellular organelles which result in reduction of sperm quality. Cryoprotective agents can minimize cryopreservation injury. Small concentrations of the synthetic polymer polyvinyl alcohol (PVA) were found to inhibit the formation of ice during cryopreservation. PVA clearly shows potent ice recrystallization inhibition (IRI) activity with arrest of ice crystal growth similar to antifreeze proteins. Therefore, the purpose of this study was to evaluate the cryoprotective effects of PVA on human sperm during cryopreservation process.
Materials and Methods: Semen samples were collected from twenty normospermic men and divided into two equal parts to be diluted with two concentrations of (0.01 % and control). Semen samples were diluted with a glycerol egg yolk citrate (GEYC) based freezing medium. Then diluted semen was cooled and packed into straws. The straws were frozen in steam of liquid nitrogen (LN2) and then preserved in the LN2 and stored until thawed and used for evaluation. After thawing, motility, viability and morphology of thawed sperm were assessed.
Results: Results showed that 0.01 % PVA improved the percentage of total motility, progressive motility and viability of human compare to control samples. Morphology was not affected by adding PVA to freezing media.
Conclusion: It can be concluded that supplementation of freezing media with PVA at concentration of 0.01%, can increase the efficiency of cryopreservation of human sperm.
Keywords: Cryopreservation, Polyvinyl Alcohol, Sperm Parameters

P-78: α- Linolenic Acid Promotes *In Vitro* Maturation and Quality of Mouse Oocytes with Polycystic Ovarian Syndrome

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Background: The maternally syntheses of mRNAs and proteins produced during oocyte growth and maturation. Therefore, the oocyte quality reflected in the stages of embryo development. In the meanwhile, polycystic ovary syndrome (PCOS) as a reproductive hormonal disorder has oocytes with poor quality under *in vitro* fertilization (IVF) cycles. In this regard, a significant improvement in oocyte survival and maturation rates will have a great potential for increasing the efficiency of IVF. The α-linolenic acid (omega-3) plays an important role in reproductive physiology, affecting oocyte quality. Therefore, this study was planned to investigate beneficial effect of α-linolenic acid (omega-3) in in-vitro oocyte maturation and oocyte quality from PCOS mice.
Materials and Methods: Female NMRI mice (25-30 day–old) were divided into two groups: i. Non-PCOS and ii. PCOS groups. PCOS mouse model induced with 4 mg/kg estradiol valerate dissolved in 0.2 mg Sesame oil once a day. After 60 days, the immature follicles were collected from PCOS ovaries, and cultured in the α-MEM medium (minimum essential medium: α-MEM) supplemented with 10% bovine serum albumin (BSA) and different concentrations of α-linolenic acid (ALA) (0 [control], 50, and 100 µM). The *in vitro* maturation of PCOS oocytes in the different doses of ALA were evaluated and compared with control and non-PCOS groups. Also, the expression of TFAM gene in mature oocytes was investigated by Quantitative Real Time PCR (QRT-PCR).
Results: In this study, the TFAM gene expression was increased during in vitro maturation of oocytes. TFAM gene was identified by QRT-PCR in all oocyte. However, the TFAM gene expression significantly increased in the MII oocytes supplemented with 50 µM ALA (95% ± 2.1% vs. 84% ± 2.1%, P=0.005), whereas the gene expression of TFAM in the oocytes treated with higher dose (100 µM of ALA) significantly decreased. Also, the rate of in-vitro maturation of germinal vesicle oocytes treated with 50 µM ALA significantly improved in comparison to control group (61/79: 77.2% vs. 59/95: 62.1%).
Conclusion: In this study, the TFAM gene expression was increased during *in vitro* maturation of oocytes. TFAM gene was identified by QRT-PCR in all oocyte. However, the TFAM gene expression significantly increased in the MII oocytes supplemented with 50µM ALA (95% ± 2.1% vs. 84% ± 2.1%, P =0.005), whereas the gene expression of TFAM in the oocytes treated with higher dose (100 µM of ALA) significantly decreased. Also, the rate of in-vitro maturation of germinal vesicle oocytes treated with 50µM ALA significantly improved in comparison to control group (61/79: 77.2% vs. 59/95: 62.1%).
Keywords: PCOS, IVM, Germinal Vesicle, α-Linolenic Acid, TFAM Gene

P-79: The Interfering Effects of IVF and Embryo Vitrification at 2-Cell Stage Upon miR-29a/29b Expressions in Mouse Blastocysts

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Background: Embryo vitrification is an important approach in assisted reproductive techniques (ART), which has improved clinical outcomes. miRNAs are a class of short single-stranded noncoding endogenous RNAs in eukaryotic cells that regulate epigenetic expressions of numerous genes at the posttranscriptional or translational levels. The present study assesses the effects of *in vitro* fertilization (IVF) and vitrification on miR-29a/29b expressions after IVF and vitrification.
Materials and Methods: Expression of miR-29a/29b in mouse blastocysts from control, IVF and vitrification groups was investigated by qRT-PCR.
Results: The levels of miR-29a/29b upregulated in the experimental groups as compared with the control group.
Conclusion: The results of this study have suggested that vitrification at 2-cell stage causes disruption in epigenetic mechanisms in blastocysts.
Keywords: IVF, miR-29a/29b, Blastocyst

P-80: The Role of Mediating Cognitive Fusion in The Relationship between Rumination and Depression in Infertile Women

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Background: One of the important areas of health of the infertile persons is a psychological aspect that requires special attention and prevention of disorders such as depression in them. "Cognitive fusion" refers to the human tendency to become entangled with thoughts as a result of strong belief in their literal content. The aim of this study was to investigate the role of mediating cognitive fusion in the relationship between rumination and depression in infertile women.
Materials and Methods: The sample consisted of 300 infertile women referred to the Royan Institute, a referral infertility clinic in Tehran, the capital of Iran. The sampling method was convenient. Four main questionnaires were used including demographic questionnaire, a patient's health questionnaire (PHQ-9), a cognitive fusion checklist (CFQ) and a rumination questionnaire (RRS-10). Data were analyzed with SPSS software. Statistics including Pearson correlation coefficient and path analysis were used to test the research hypotheses.
Results: The results of this study showed that there was a significant positive correlation between cognitive rumination and cognitive fusion ($P < 0.001$, $r = 0.611$), rumination and depression ($P = 0.001$, $r = 0.336$) and cognitive fusion and depression ($P < 0.001$ and $r = .5881$). Conceptual model of research and standardized coefficients.
Conclusion: In general, it can be concluded that cognitive fusion and rumination are factors affecting depression in infertile women. Therefore presence of psychologists and psychiatrists in diagnostic and treatment centers of infertility is necessary in order to identify the risk factors and to educate the correct ways of dealing with feelings of inadequacy and depression through their expertise.
Keywords: Cognitive Fusion, Rumination, Depression, Infertility

P-81: Direct Effect of Controlled Osmotic Stress on Nuclear

Maturation of Bovine Oocytes

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Background: Recent studies have indicated that a well-defined and properly applied stress treatment of oocytes may induce general their adaptation, improve survival, and in vitro development. The current study was designed to understand the effect of controlled and sublethal osmotic stress on nuclear in vitro maturation of bovine oocytes as a first step toward improving tolerance to various in vitro procedures such as cryopreservation, enucleation and SCNT.
Materials and Methods: Bovine immature aspirated oocytes from abattoir-derived ovaries were initially cultured in isosmotic IVM medium (bicarbonate-TCM 199 supplemented with 10% FCS and 0.1IU/ml FSH) (osmolality was 280-285mOsm) for 1 hours adaptation. After that, the oocytes of treatment group were exposed to hyperosmotic medium (500-510 mOsm IVM medium contained sorbitol) for 4 hours and then transferred to IVM medium. All of the maturation procedure was carried out at 38.5ºC with a 5% CO_2/air atmosphere for 22-24 hours. Eventually nuclear maturation of oocytes was evaluated with 5 µg/ml H33342 for 5 minutes under an epifluorescent microscope.
Results: The results showed that exposure of bovine oocytes to high osmolality had not any effect on nuclear maturation potential of osmotic stress-treated oocytes [(12.05% MI stage and 86.14% MII stage versus 11.36% MI stage and 84.42% MII stage for control and treatment groups, respectively)] (P>0.05).
Conclusion: Treatment with sublethal doses of high osmotic stress couldn't induce any detrimental effect on in vitro maturation ability of bovine oocytes and oocytes could tolerate hyperosmotic stress for up to 4 hours. In this study osmotic stress was carried out in the first quarter of bovine oocyte maturation period wherein the transcription potential of oocyte is still active. Accordingly, this controlled osmotic stress can induce efficient changes in the gene expression programs by intracellular signaling networks and may outline a completely new strategy in mammalian embryology.
Keywords: Osmotic Stress, Nuclear Maturation, Bovine, Oocytes

P-82: Extended Incubation of Mouse Spermatozoa Affects The Preimplantation Embryonic Development

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Background: Sperm quality is a determinant factor on the outcome of assisted reproduction and is influenced by many factors, such as time and temperature during in-vitro incubation of sperm. This study investigated the effects of long-term in vitro sperm incubation at room temperature (RT) on Sperm quality parameters and early embryonic development.
Materials and Methods: Mouse sperm samples were divided in to 2 groups (fresh and incubated at RT for 24 hours). sperm parameters were assessed according to WHO guidelines. DNA Fragmentation index (%DFI) was evaluated by SCSA. Mouse sperm were microinjected into mouse oocytes. Fertilization rete and embryo development were evaluated.
Results: The percentage of progressive motility significantly decreased in samples incubated at RT compared with fresh group (P <0.05). The percentage of DFI was not significantly altered in samples incubated at RT compared with fresh group (P>0.05). The morula and blastocyst rate significantly decreased after 24 hours incubation at RT (P <0.05).
Conclusion: The result of this study demonstrated that extended sperm incubation affects preimplantation embryonic development.
Keywords: Sperm Incubation, Embryo Development, DNA Fragmentation

P-83: Evaluation of N-acetylcysteine (NAC) Effect on *In Vitro* Culture of Immature Mouse Testis following Vitrification

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Background: Cryopreservation of testicular tissue in order to preserving spermatogonial stem cells (SSCs) is a suitable method can be provided to prepubertal boys at the risk of infertility due to cancer treatments. Cryopreserved tissue can be transplanted to the individual or cultivated in the laboratory. Due to the culture time, tissue damage is high, so optimizing the culture condition is essential. In this study, the effects of NAC presence in the culture medium of vitrified mouse testicular tissue will be investigated and cell viability will be evaluated.
Materials and Methods: Testis tissue were harvested from sacrificed immature NMRI male mice and vitrified (freezing medium: DMSO, Ethylene Glycol, DMEM, 20% FBS) After warming, testis tissues were cultured in vitro for 7 days on agar gel in a medium composed of RPMI, $NaHCO_3$ and 10% KSOR. Culture medium were supplemented by different dosages of NAC (0, 5, 10, 20, 25, 37.5, 50 mmol/L). Following 7 days of culture, cell viability were evaluated by Flow cytometry method.
Results: Our results showed that cell survival At the end of the culture period was 54% at a NAC concentration of 50 mmol/L, 43% at 37.5 mmol/L, 39% at 25 mmol/L, 33% at 20 mmol/L, 22% at 10 mmol/L and 29% at 5 mmol/L. Cell survival was significantly higher in a group cultured without NAC compared to the groups cultured in presence of NAC (P<0.005).
Conclusion: Although, our results showed that the used concentrations of NAC wasn't effective enough to suppress cell death in *in vitro* culture of vitrified testes, previous researches established the ability of NAC to inhibit apoptosis in testicular germ cells *in vitro*. Thus, it seems that higher concentrations of this antioxidant should be tested.
Keywords: Cryopreservation ,Testicular Tissue, NAC, Cell Vi-

ability

P-84: Protective Effect of Misoprostol on Ibuprofen-Induced Alteration of Testes Stereological Structure in Adult Male Mice

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Background: Misoprostol, a prostaglandin E1 analog, is used clinically during treatment of duodenal and gastric ulcers with non-steroidal anti-inflammatory drugs such as ibuprofen to inhibit acid secretion and stimulate bicarbonate and mucus secretion. It is well known that long time administration of ibuprofen can reduce sperm parameter and testosterone level but there is no study about effect of ibuprofen on structure of testis. This study was conducted to evaluate the structure of testis after long time administration of ibuprofen and protective effect of misoprostol on ibuprofen-induced alterations in testis tissue of adult male mice.
Materials and Methods: In this study, 80 adult male mice were divided into 8 groups including: control, ibuprofen treated group which received 6 mg/Kg/day ibuprofen, three misoprostol treated groups which received 1, 10 and 100 µg/Kg/day misoprostol and three ibuprofen+misoprostol treated groups which received 6 mg/Kg/day ibuprofen with 1, 10 and 100 µg/Kg/day misoprostol. All treatments were carried out for 40 consecutive days by oral gavage. At the end of experiment, animals were euthanized and their left testis were removed and fixed in 10% neutral buffered formalin. Tissues were processed by standard paraffin embedding and serially sectioned at 20 µm thickness. Twenty to twenty-five selected sections by systematic random sampling were stained with HandE and total volume of testis, germinal epithelium, interstitial tissue, and total numbers of spermatogenic, Sertoli and Leydig cells were estimated by point counting method based on Cavalieri's principle and optical dissector technique using by stereo-investigator software. Finally, one-way ANOVA and Tukey's post-hoc were performed for data analysis.
Results: Results showed that ibuprofen reduced total volume of germinal epithelium and total number of all spermatogenic cells, Sertoli cells and Leydig cells and also increased interstitial tissue significantly compared to control group. Our results indicated that misoprostol could inhibit the ibuprofen-induced alterations in a dose dependent manner. Stereological studies revealed that misoprostol increased total volume of germinal epithelium of seminiferous tubules and total number of spermatogenic cells, Sertoli and Leydig cells as well as decreased total volume of interstitial tissue significantly compared to ibuprofen treated group.
Conclusion: Based on our results, it can be concluded that administration of misoprostol can improve structure of testis after ibuprofen administration and can be considered as a suitable protective strategy for improvement of male infertility or subfertility due to NSAIDs administration.
Keywords: Misoprostol, Prostaglandin E, Ibuprofen, Testis, Stereology

P-85: The Effect of Sperm DNA Fragmentation on Parameters of Sperm, Blastocyst Formation , Embryo Quality and Pragnance Rate in Assisted Reproductive Technique

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Background: The aim of the present study was to investigate the correlation between sperm DNA fragmentation standard laboratory and parameters of human semen. We have also examined an impact of sperm DNA damage on embryo quality and Blastocyst Formation after ICSI procedures.
Materials and Methods: Semen samples from 50 men have been analyzed as a part of diagnostic semen analysis and fertility evaluation by using conventional microscopic semen analyses. In each sample, the concentration, motility, morphology and vitality of spermatozoa were evaluated. Sperm DNA damage was determined by using Halo sperm test with threshold value of DNA fragmentation index (DFI) at (<15%, 15-20% ,>30 %). Embryo quality was evaluated undergoing assisted reproduction technique (ART) procedures at our clinic.
Results: The results of this study indicate that the patient group with DFI ≥ 30 % had significantly lower values of standard semen parameters , Blastocyst Formation and embryo quality than the group of patients with DFI index(<15%,15-20%). In addition Negative correlations were found between DNA fragmentation and motility (r= -0.41, P<0.001), morphology (r = -0.32, p = 0.001) and vitality (r= -0.36, P<0.001). We also found that in the group of patients with DFI index (<15%,15-20%) the were significantly more embryos of better quality.
Conclusion: In men with poorer semen quality, evaluated by standard semen parameters, a higher proportion of sperm with damaged DNA can also be expected. Higher sperm DNA damage, established by Halos perm test, also had an impact on embryo quality in this group of patients.
Keywords: DNA Fragmentation, Parameters Sperm, Blastocyst Formation

P-86: Effect of Royal Jelly in The Presence of Folic Acid Supplement on NMRI Mouse Sperm Count

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Background: About 40 percent of infertility factors are related to men, which can affect a person's job, body, personality, and mentality. Royal jelly is a protein secretion from the Hypopharynx and the lower jaw glands of the worker bee. Only the food given to it during the lifetime of the queen bee and all the small larvae are fed with royal jelly. Extensive combinations of it, as well as many properties like the antioxidant properties of this material have been proven. That's why we chose this material to measure its effects on the sperm count, as well as in the presence of Folic acid supplementation, which is important in the quality of male fertility.
Materials and Methods: In this study, NMRI mouse weighing approximately 20 grams were used. Royal jelly at doses of 0.1,

0.2 mg/Kg and Folic acid, at doses of 0.08 , 0.15 mg/Kg was injected intraperitoneally for 15 days. The cauda epididymis was removed by surgery and transferred to MHRM medium and studied after incubation.
Results: The results showed that the sperm count in the Royal jelly (0.1 mg/kg) and Folic acid groups (0.08 mg/kg) significantly increased compared to the control group. Other groups did not increase significantly.
Conclusion: According to the results of this study, Royal jelly has increased the sperm count, and the use of Folic acid as a supplement to it further increases the sperm count and male fertility. The results of this study can be used in the pharmaceutical industry to produce drugs to improve male fertility.
Keywords: Royal Jelly, Folic Acid, Sperm Count, Sperm Quality

P-87: Increase Expression of IL-1b Transcriptin Cultured-PBMCs

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Background: There is strong evidence that cytokines and growth factors play an important role as local mediators of the actions of steroids on the endometrium to prepare it for implantation. In humans, PBMCs were reported to induce the production of several cytokines, inflammatory cytokines such as IL-1b which play a key role in primary immune responses. In this article, the impact of intrauterine administration of PBMCs on the expression of il1-b transcript and role il1-b in embryo implantation and pregnancy rate.
Materials and Methods: Pregnant mice were randomly divided into two groups, including embryo implantation dysfunction (EID) group; EID with PBMCs group. Mouse PBMCs were isolated from whole blood of the non-pregnant female mouse and then cultured 0, 24, or 48 hours *in vitro*. Uterine horns were excised to determine the number of pregnant mice and implantation sites on the Day 7.5 postcoitum. mRNA expressions of interleukin-1b (IL-1b) in the cells were examined using the quantitative real-time polymerase chain reaction analysis (real-time PCR).
Results: IL-1b transcript was expressed in mouse cultured-PBMCs. PBMCs significantly increased IL-1b ($P < 0.05$) mRNA level in mouse PBMCs. This result showed that the expression of IL-1b mRNA in mouse PBMCs was transiently increased.
Conclusion: Intrauterine administration of mouse PBMCs derived from unpregnant mice prior to embryo implant have a good influence on endometrial receptivity and embryonic implantation in EID mice.
Keywords: Embryo Implantation Dysfunction, Peripheral Blood Mononuclear Cells, Endometrial Receptivity,il-1b, Mouse

P-88: Supplementation of Beltsville Extender with Plant-Origin Cryoprotectant for Cryopreservation of Rooster Semen

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Background: The avian sperm is susceptible to sever damages during the freeze-thaw process due to high sensitivity to cold shock. Integrity of the rooster sperm membrane should be protected during freeze-thaw process. Extenders play an important role to reduce detrimental effects of freezing on sperm due to their stabilization effects on the sperm plasma membrane. Soybean lecithin is a novel plant-origin cryoprotectant that can protect sperm during freeze- thaw process. This cryoprotectant has not been assess in combination with Beltsville extender for rooster semen freezing. The aim of present study was to assess rooster sperm parameters after cryopreservation in Beltsville extender supplemented with different concentrations of soybean lecithin (SL) compared to Beltsville extender supplement with different concentrations of egg yolk (EY).
Materials and Methods: Semen samples were collected twice a week from ten roosters. Then samples were pooled to eliminate individual difference and subsequently divided into 5 equal parts to be diluted with Beltsville Extenders containing different cryoprotectants as follow: 0.5% SL, 1% SL, 5% EY, 10% EY and 15 % EY. The diluted semen was gradually cooled to 4 °C for 3 hours. Then cooled semen was loaded into 0.5-mL straws and they were exposed to liquid nitrogen vapor for 10 min, plunged into liquid nitrogen (LN2), and stored LN2 until thawed and used for evaluation of sperm parameters.
Results: The highest significant percentage of total and progressive motility were obtained in Beltsville supplemented with 1% SL ($P<0.05$). 0.5% SL produced the lowest significant percentage of motility and viability ($P<0.05$). Between extenders containing egg yolk, 15% egg yolk produced the higher motility and viability of thawed sperm compared to 5% and 10% EY.
Conclusion: It seems that replacement of egg yolk by soybean lecithin in Beltsville extender may produce the higher quality of frozen-thawed semen. According to the result, lecithin at concentration of 1%, improves the motility and viability of rooster semen during cryopreservation.
Keywords: Cryopreservation, Phospholipids, Antioxidant

P-89: Effect of Freezing–Thawing Process and Quercetin on Sperm Survival and DNA Integrity in Patients with Asthenospermia

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Background: Sperm cryopreservation is an important component of fertility management and much of its successful application seems to affect the reproductive outcome of assisted reproduction technologies (ART): appropriate use of cryoprotectants before and sperm selection technologies after cryopreservation seem to have the greatest impact on preventing DNA fragmentation, thus improving sperm cryosurvival rates. Quercetin is a flavonoid with high reactive oxygen species (ROS) scavenging and ion chelating activity. We were interested in testing the effect of quercetin, as an antioxidant, in preventing sperm damage during the freeze-thawing process.

Materials and Methods: Spermatozoa from 20 patients with asthenospermia were incubated in vitro with 50 µM quercetin or phosphate-buffered saline as a control for 1 hours. After this incubation period and administration of these compounds during freezing process, sperm progressive motility, sperm morphology, viability and DNA integrity were assessed before and after freezing/thawing process. Sperm motility and count were assessed according to WHO criteria, eosin nigrosin assay to assess sperm viability and the acridan orange assay to assess sperm DNA integrity, and papanicolaou assay to assess sperm morphology. Statistical analysis was performed by the student's t test.
Results: Data showed that supplementation of the cryopreservation medium with quercetin induced a significant improvement in post thaw sperm parameters, compared to those of control, regarding sperm morphology ($p = 0.05$), viability ($P=0.001$) and DNA integrity ($p = 0.05$).
Conclusion: Quercetin could have protective effect during cryopreservation and improves the quality of cryopreserved human semen but further research is needed to confirm this effect.
Keywords: Quercetin, Cryopreservation, DNA Integrity, Sperm Viability, Asthenospermia

P-90: Effects of L-Carnitine and Fibrin Encapsulation on In Vitro Maturation and Oocyte Developmental Potential Obtained from Transplanted Mouse Ovarian Tissues

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Background: Ovarian tissue transplantation is emerging as a powerful approach for preserving fertility for women that are losing ovarian function. In addition, I vitro maturation of oocytes retrieved from grafted ovaries may overcome the fertility defects in some cases. The objective of this study was to evaluate the potential of using L- Carnitine (LC) as an antioxidant and fibrin encapsulation to improve developmental potential of oocytes obtained from grafted ovarian tissues.
Materials and Methods: NMRI mice were divided into six groups: Control (non-graft), Transplant (autograft), Saline group (autograft + saline), LC group (autograft + LC), Fibrin group (autograft + fibrin), LC + Fibrin (autograft + LC + Fibrin). 6- weeks- old mice were ovariectomized and left ovaries were transplanted into the back muscle tissue. LC (200 mg/Kg) was injected intraperitoneally one day before surgical operation and repeated until one week after grafting. On surgical day, Tissues were encapsulated in fibrin and transferred into the back muscle. Three weeks later, ovarian grafts were recovered and oocytes were harvested for *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* development (IVD).
Results: Our results indicated that the number of retrieved immature oocytes as well as successful IVM, IVF and IVD in transplanted groups was significantly lower than control group ($P<0.05$). All transplanted groups contained some oocytes that survived following IVM, IVF and IVD and no significant difference was seen between grafted groups.
Conclusion: Our study demonstrate that LC and fibrin scaffold did not show any negative effect on transplants but could not support further development of oocytes. It seems that usage of scaffold in combination with a growth factor could improve autotransplantation results and more studies are needed in this area.
Keywords: Ovarian Transplantation, L-Carnitine, Fibrin , IVF

P-91: Effect of Melatonin on the Number and Quality of The Egg and The Number of Embryos in The Antagonist Intracytoplasmic Sperm Injection Cycle

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Background: In women with a history of intracytoplasmic sperm injection (ICSI), this study was conducted to investigate the effect of melatonin on the number and quality of the egg and the number of embryos in the antagonist cycle. The primary focus of our present study was to evaluate the influence of Melatonin on oocyte and embryos quality.
Materials and Methods: A group of 100 female infertility clients from Qom Infertility center Acecr were randomly selected and divided into two groups. Group 1 received melatonin 3mg and group 2 received matching placebo capsule. At the same time as the start of gonadotropin drugs, the group1 was given a melatonin capsule at night and continued until the oocyte retrieval day.
Results: According to the findings of the study, the average number of adult oocytes (MII) in group1 was more than group 2(88.5 vs. 56.4) and this difference was significant. Also, there was a significant difference between the mean number of immature oocytes (MI) in both groups .
Conclusion: Despite the positive effect of melatonin on the number of MII oocytes and the improvement in the quality and number of mature oocytes, Melatonin has no significant effect on the number of embryos during antagonist cycle.
Keywords: Melatonin, Oxidative Stress, Infertility

P-92: Protective Effect of Heparin on Blood Antioxidant Enzyme Activities in Ischaemic-Reperfusion Injury of The Rat Testicle

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Background: Testicular torsion is an urologic emergency that causes testicular damage and lead to reduced fertility or infertility. It appears that the main pathophysiology of testicular torsion is ischemia/reperfusion (I/R) injury of the testis caused by the twisted spermatic cord and its release. Surgical detorsion is currently the only treatment and allows blood reperfusion. these ischemia-reperfusion injuries are associated with over production of reactive oxygen species (ROS). Many antioxidants and free radical scavengers have been proposed in recent years for treatment of testicular torsion-induced male infertility. Heparin is a naturally occurring anticoagulant produced by basophils

and mast cells.
Materials and Methods: Eighteen Wistar Albino male rats weighing 250–300 g were divided into three groups: sham (group S, n = 6); torsion/detorsion (group T/DT, n = 6), and heparin pretreatment (group Hep, n = 6). The left testes were rotated 720° clockwise for 2 h in the rats of the torsion– detorsion group (group T/DT). Rats in the treatment group underwent the same surgical procedure as the torsion–detorsion group but were also given heparin 800 IU/kg (Hep group) by an intraperitoneal route 30 min prior to detorsion. In sham group (group s), the left testes were brought out through the incision and were placed back in the scrotum. After 2h of reperfusion 5ml intracardiac blood samples were taken.Blood plasma malondialdehyde (MDA), Glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (TAC) activities, and levels were measured.
Results: Heparin significantly reduced MDA and increased CAT but GPX and TAC did not differ significantly in Hep group in comparison with T/DT group.
Conclusion: Our results suggest that heparin treatment has a protective role on I/R-induced testicular injury.
Keywords: Testicular Torsion, Antioxidant Activity, Heparin, MDA, CAT

P-93: Inflammatory Alterations of Testicular Tunica Albuginea in Adult Male Rats under Different Temperatures Induced Heat Stress

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Background: Heat stress (HS)-induced testicular damage is one of the infertility causes in men and most mammals. Even short-term exposure of the testicles to the heat can result in spermatogenesis arrest.
Materials and Methods: Twenty-eight male rats were randomly categorized into 4 groups; group 1 served as a control, groups 2, 3 and 4 were heat-stressed (37, 39 and 43°C for 20 minutes per day, respectively). The HS was induced through immersion of experimental rat scrotums in a water bath. After 35 days, the testicles were harvested and inflammatory alterations of testicular tunica albuginea were analyzed in all experimental groups.
Results: In HS-exposed groups, testicular tunica albuginea inflammation rate showed significant increase in a temperature-dependent manner compared to the control group.
Conclusion: The HS can cause negative changes in testicular histo-architecture in a temperature-dependent manner.
Keywords: Heat Stress, Testis, Tunica Albuginea, Rat, Inflammation

P-94: Evaluation of Oxidative Stress and Malondialdehyde Levels after Recombinant FSH (Gonal F) Treatment in Oligozoospermia Infertile Men

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Background: FSH plays an important and essential role in spermatogenesis, maintaining and integrating the DNA sperm. Several studies showed that infertile men with normal semen parameters have low levels of stress oxidative while infertile men with abnormal semen parameters have more stress oxidative level. The aim of this study was to evaluate whether the administration of recombinant FSH (Gonal-f) could improve sperm parameters. The secondary endpoints of this study were to evaluate stress oxidative and Malondialdehyde Levels in oligozoospermia infertile men.
Materials and Methods: In the present study, an interventional study was carried out with a sampling of 50 infertile men (oligozoospermia). The semen samples were examined in accordance to the WHO (2010) criteria. Then before and after treatment with rhFSH (Gonal F) sperm parameters was determined by light microscopy and oxidative stress were measured with flowcytometry assay. Also Malondialdehyde Level was evaluated with TBA assay.
Results: Measurement of sperm parameters (concentration, motility and morphology) in oligozoospermia patients before and after rhFSH treatment was significantly different and improved ($P<0.001$). Also, the oxidative stress and Malondialdehyde Levels of seminal plasma significantly decreased after rhFSH treatment ($P <0.05$).
Conclusion: According to the above results, rhFSH treatment has a beneficial effect on sperm parameters in oligozoospermia males and dramatically reduces oxidative stress. Also, increasing Malondialdehyde level could be a negative effect of oxidative stress on sperm quality and performance. In this study, Malondialdehyde Level also showed a significant decrease after treatment.
Keywords: Oxidative Stress level, Malondialdehyde level, FSH Treatment, Oligozoospermia

P-95: Fennel and Cinnamon Combined Extract Improve Spermatogenesis and Protect Testis Tissues in Busulfan Induced Infertile Rats

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Background: Busulfan is one of the common cancer treatment drugs with infertility side effect, fennel and cinnamon are two medicinal plants with fertility enhancement properties. The aim of this study is to investigate the effects of fennel and cinnamon on busulfan induced infertile rats.
Materials and Methods: 40 male wistar rats were divided into 4 groups including: sham group: healthy rats without intervention, control group: Busulfan treated rats, fennel group: busulfan and fennel extract treated, fennel and cinnamon group: busulfan, fennel and cinnamon extract treatment. Testicular tissues were sampled and the testicular physical parameters and spermatogenesis level were evaluated by H and E staining and optical microscopy imaging.
Results: The biggest and the smallest length testis were observed in cinnamon + fennel and fennel groups respectively ($P <0.05$). The highest and lowest sperms level was observed in the cinnamon + fennel group and fennel group respectively ($P <0.001$). The total average of reproductive cells in the cinnamon + fennel group was the most (104.17) and in control group was in least level .
Conclusion: The combined extract of fennel and cinnamon

significantly protect the testicular tissues against infertility effect of busulfan, however the fennel extract alone increased the busulfan effect in rat.
Keywords: Fennel, Infertility, Cinnamon, Rat, Busulfan

EP-96: Enrichment of Human Spermatogonial Cells by Culture of Testicular Cell Suspension in Obstructive Azoospermic Patients

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Background: 50 million of men are infertile, and notably, azoospermia comprises 25% of male infertility cases. Therefore, it is of great interest to generate functional gametes for patients with male infertility especially for azoospermia. Spermatogenesis is a complex process regulated by multiple interactions between developing germ cells and the surrounding microenvironment. Production of sperm in vitro would not only provide male gametes for azoospermic patients but also offers various methods for the in vitro derivation of male germ cells have been developed. So, in the present study, we investigated different methods to proliferation of SSCs in obstructive azoospermic patients.
Materials and Methods: Human testicular samples were obtained from men with obstructive azoospermia. after enzymatic digestion process, cells assigned to following groups: culture of SSCs in the dish without cover (control group), co-culture of SSCs with infertile sertoli cells (I), co-culture of SSCs with fertile sertoli cells (II), culture of SSCs on nanofiber (covered with laminin) (III), culture of testicular cell suspension (IV). Then cells were cultured for two weeks in 34-StemPro medium and evaluated colony formation of human spermatogonial cells and gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, Oct-4) and specific germ cell (Integrin α6, Integrin β1, PLZF) genes in five different culture systems.
Results: We found that the highest number and diameter of colonies and cellular proliferation associated with IV group which were significantly different with control group and other groups, while it was fewest in control group, III, II, I groups respectively. Expression of germ specific genes in IV group were significantly increased ($P \leq 0.05$) and levels of expression of pluripotency genes were significantly decreased in this group ($P \leq 0.05$) compared with other groups. Gene specific methylation pattern of examined genes did not show any changes during culture period in culture systems.
Conclusion: Our findings indicate that testicular cell suspension can reconstruct a microenvironment capable of regulating proliferation of cell colonies.
Keywords: Spermatogonial Stem Cells (SSCs), Colonization, Culture Systems, Obstructive Azoospermia

P-97: The Hormonal Effect of The Artificial Sweetener (Acesulfame Potassium) on Adult Male Rats

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Background: Acesulfame potassium is a low-calorie artificial sweetener. It is important to know its possible health effects because of high consumption of acesulfame potassium. Therefore, this study aimed to investigate effect of the intraperitoneal administration of acesulfame potassium in pregnancy and after lactation on adult male offspring sexual and gonadotropin hormones.
Materials and Methods: In this study, acesulfame potassium with four different doses, 50 g, 100 g, 200 g and 400 g/kg body weight (BW) was injected intraperitoneally to pregnant rats (wistar) and then to their adult offspring after lactation until 60th. Blood samples were also collected after puberty and the levels of FSH, LH and testosterone were measured by using Elisa method. Also, mother's rats and their offspring were weighted during study. ANOVA was used to compare data between groups.
Results: The mean weight in both treatment groups with concentrations of 50 and 200 mg/kg was significantly higher than that in controls and the other groups. There was no statistically significant difference in LH levels between different groups. Administration of acesulfame potassium significantly reduced FSH levels in neonates who received two different doses of 50 mg and 100 mg/kg BW ($P<0.01$). Testosterone level was significantly ($P<0.001$) increased in 50 g/kg 200 g/kg and 400 mg/kg as compared with controls.
Conclusion: It seems that the use of acesulfame potassium as an artificial sweetener in food products and its use by pregnant mothers and infants probably have hormonal effects, although there is no evidence that this alteration causes spermatogenesis changes.
Keywords: Acesulfame, Potassium, Pregnancy, Testosterone, Male Rat

Female Infertility

P-98: Sitagliptin Significantly Improves The Maturation of Oocyte and Embryo Quality More Than Metformin in Infertile Polycystic Ovary Syndrome Patients

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Background: Polycystic ovarian syndrome (PCOS) is one of the pathological factors involved in low-quality oocytes, embryos and the failure of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Studies have shown improving metabolic–endocrine factors in these patients may increase

the quality of oocytes and embryos. On the other hand, there is a growing interest in the use of insulin sensitizer drugs and type 2 diabetes in the treatment of PCOS and researches in recent years have shown that metformin and sitagliptin has been reported to improve ovarian cycles and ovulation in PCOS patients. We aimed to compare the effects of metformin and sitagliptin on oocytes and embryo quality in PCOS individuals undergoing ICSI.

Materials and Methods: In this clinical trial, 60 infertile PCOS patients were selected based on the Rotterdam criteria; Then they were divided into 3 groups (n=20): metformin group (Glucophage, Merck, West Drayton, UK; 500 mg, twice a day), sitagliptin group (Januvia, Merck, West Drayton, UK; 50 mg, twice a day) and placebo group. All patients were undergoing treatment with antagonist GnRH protocol. Treatment was carried out two months before the start of the ovulation cycle and continued until the day of oocyte aspiration. Mature (MII) oocytes were identified by the presence of the first polar body under a stereomicroscope (Olympus, Tokyo, Japan). Only those oocytes that had extruded the first polar body (MII oocyte) were used for ICSI. The number of immature oocytes [(Germinal vesicle (GV) and metaphase I (MI)] were counted. Four hours after oocyte retrieval, a single motile sperm with an apparently normal morphology was immobilized and used to inseminate the oocyte. Fertilization was assessed the next day by the presence of two pronuclei (2PN). Embryo quality was assessed on the 3rd day of insemination and graded as follows: Grade I, symmetric blastomeres and no fragmentation; Grade II, unequal blastomeres and <30% fragmentation; and Grade III, unequal blastomeres and >30% fragmentation.

Results: The number of mature oocytes in the metformin (9.86 ± 3.11vs. 6.20 ± 3.83; P=0.04) and sitagliptin (10.06 ± 4.86 vs. 6.20 ± 3.83; P=0.03) groups increased significantly in comparison with the placebo group. While the number of immature oocytes decreased significantly in the sitagliptin group compared with the placebo group (1.46 ± 1.18 vs. 3.66 ± 0.97; P=0.001), it also revealed a significant reduction in the sitagliptin group compared with the metformin group (1.46 ± 1.18 vs. 2.60 ± 1.50; P=0.04). The number of grade I embryos increases significantly in the sitagliptin group compared to the placebo (3.13 ± 1.35 vs. 1.93 ± 1.03, P=0.010) and metformin (3.13 ± 1.35 vs. 2.06 ± 0.70, P=0.024) group. There were no significant differences in the total number of retrieved oocytes, fertilized oocytes, number of grade II and III embryos among the groups (P>0.05).

Conclusion: We conclude that sitagliptin can improve the maturation of oocyte and the quality of embryos more effectively than metformin, in PCOS patients undergoing ICSI.

Keywords: Sitagliptin, Metformin, Polycystic Ovary Syndrome, Intracytoplasmic Sperm Injection

P-99: GM3-Synthase (hST3Gal V) Gene Expression in Endometrial Tissues of Women with Endometriosis

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Background: Endometriosis is a gynecological disease, affects 10-15% of women in their reproductive ages under the influence of hormonal, genetic, epigenetic, and environmental factors. According to the Sampson's theory, endometrial cells are implanted and proliferated outside the uterine cavity, attacking the pelvic structures and causing chronic inflammation. Hence endometriosis can be considered as a benign cancer. Changes in the cell surface glycosylation is a common phenotype observed in cancers that is associated with the potential for cancer cell metastasis and invasion. Studies is indicative of changes in the expression of the hST3Gal V gene, which encodes the GM3 synthase enzyme (the producer of the GM3 ganglioside). In this study, we examined changes of hST3gal V gene expression in ectopic and eutopic endometrial tissues of women with endometriosis compared with control group.

Materials and Methods: Samples were collected from 10 patients with endometriosis (5 in each group) and 5 as control group. Ectopic biopsies were obtained with the use of laparoscopic procedure, eutopic and control biopsies were obtained with the use of pipelle. RNA extraction and cDNA synthesis were performed for all samples and then gene expression was measured by Real time-PCR method.

Results: Results showed that the hST3Gal V gene expression was reduced in endometriotic tissue samples in compare to control group.

Conclusion: It seems that the lower expression of hST3Gal V gene in endometriosis is involved in the etiology of the disease.

Keywords: Endometriosis, hST3Gal V, Ganglioside, Gene Expression

P-100: Royal Jelly Improved Hormonal Changes in Rat Model of Polycystic Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is an endocrine and complex metabolic disorder which associates with anovulation, changes of biochemical factors and sex hormone, and ovarian tissue changes. Royal jelly (RJ) has modulators of sex hormone and anti-oxidant properties. The aim of present study was to examine the RJ therapeutic effect on PCOS related hormonal and biochemical changes in a rat model of PCOS.

Materials and Methods: In this study, 42 female Wistar rats (180-200 g) were divided into six groups (n=7); control, PCOS, RJ (100 mg/kg), RJ (200 mg/kg), PCOS+RJ100 and PCOS+RJ200 mg/kg. After 21 days, the animals were weighted

and dissected. The serums were used for nitric oxide (NO) and ferric reducing antioxidant power (FRAP) assay and estradiol (E2) and progesterone measurements. The ovaries were assessed for histological changes.
Results: PCOS decreased progesterone and FRAP levels and increased E2 and NO levels. In RJ-treated PCOS groups, progesterone (P=0.01) and FRAP levels (P=0.00) increased, and E2 (P=0.00) and NO (P=0.00) levels significantly decreased. The number of mature follicles (P=0.01) and corpus luteum increased significantly (P=0.00).
Conclusion: RJ improved PCOS changes of biochemical and reproductive parameters by antioxidant properties
Keywords: Royal Jelly, Polycystic Ovary Syndrome, Ovary, Sex Hormone

P-101: Culture of Post Thaw Cleavage Stage Embryos to Blastocyst Significantly Improves Clinical Outcomes of Frozen Thawed Embryo Transfer Cycles

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Background: According to the results of previous studies reporting the better reproductive outcome following blastocyst transfer in assisted reproductive technology (ART) cycles, there is a shift from early cleavage stage embryo transfer to blastocyst transfer. The aim of this study was to compare reproductive outcome following transfer of thawed cleavage stage embryos and blastocysts derived from frozen-thawed cleavage stage embryos.
Materials and Methods: In this randomized controlled trial study, which was conducted between November 2015 and June 2020, a total of 182 women aged ≤37, undergoing frozen-thawed embryo transfer (FET) cycle were included. Patients were excluded from the study if they had previous surgery on the uterus and ovaries, history of recurrent abortion, uterine or sever male factor infertility and diminished ovarian reserve. Patients were randomly assigned to one of the two groups of thawed cleavage embryo transfers (n=110) and transfers of blastocysts from extending culture of post thaw cleavage stage embryos (n=72). Clinical pregnancy rate was primary outcome measure.
Results: There were no statistically significant differences between groups with regard to mean age, body mass index, infertility duration and endometrial thickness on embryo transfer day. The rates of clinical pregnancy (56.9% vs. 40.9 %; P=0.034), implantation (34.4% vs. 19.8 %; P=0.001), ongoing pregnancy (50.0% vs. 36.6 %; P=0.028) and live birth (49.3% vs. 33.6 %; P=0.036) were significantly higher in transfer of blastocysts from extending culture of post thaw cleavage stage embryos than thawed cleavage embryo transfers. There were no statistically significant differences in respect to rates of miscarriage and multiple pregnancy in two groups.
Conclusion: These data suggest that in FET cycles, transfer of blastocysts from thawed cleavage embryos seems to produce better reproductive outcomes than thawed cleavage embryo transfers. A further large clinical trial study design is suggested.
Keywords: Blastocyst, Cleavage Embryo, Frozen-Thawed Embryo Transfer

P-102: Knowledge of Polycystic Ovary Syndrome in High School Girls' Students and Their Mother in Tehran

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Background: Polycystic Ovary Syndrome (PCOs) is one of the most common endocrine disorders in women during reproductive age. The prevalence of PCOs has been reported from 2.2% to 26% in different countries. Women with PCOs are at risk for reproductive problems such as infertility, endometrial cancer, late menopause, and metabolic disorders, including insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular disease. So, this study is conducted to assess the knowledge of PCOs among the high school girls' students and their mothers.
Materials and Methods: This cross-sectional study was carried out on 1580 high school girl's student and 480 their mothers in Tehran, capital of Iran from August to October 2017. We considered Tehran as 5 geographic regions (north, south, east, west and center) and selected schools from each of these regions randomly. Students and their mothers answered a self-administered questionnaire about knowledge of PCOs separately. Statistical analyses were carried out with R version 3.2.1. Main analyses were multilevel analyses.
Results: In this study, the average age of students and their mothers were 16.97 ± 0.84 (Mean ± SD) and 45.19 ± 5.02, respectively. The results of this study showed that only 48 student (3%) and 160 mother (33%) had knowledge about PCOs. 25.31% of girls got their information about PCOs from their mothers, 25% from internet, 18.75% from health educator, 18.75% from radio and television and 14.58% got information about PCOs from their friends. The results of this study showed that knowledge of student about PCOs was directly and significantly related to mother's knowledge about PCOs (P<0.001).
Conclusion: The results of this study indicate that girls and mothers' knowledge of the causes, symptoms, diagnosis, and treatment of PCOs is not sufficient. Due to the heavy load and significant impact of PCOs on reproductive and public health of Iranian women, health authorities should plan to raise the awareness of girls and women about its symptoms and complication in the community.
Keywords: Knowledge, Polycystic Ovary Syndrome, Girls' Students, Mother

P-103: Co-Culture of Granolosa Cells Near Immature Oocyte in Intracytoplasmic Sperm Injection and In Vitro Fertilization

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Background: To consider which methods of micromanipulation techniques increases the embryo quality of human oocytes cultured with or without cumulus cells (CCs), we aimed to compare intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF).
Materials and Methods: Five hundred fifty immature oocytes were retrieved and were randomly divided into two groups; oocytes that were cultured with CCs (Group A) and oocytes cultured without CCs (Group B). After in vitro maturation (IVM), only oocytes that displayed Metaphase II (MII) stage went randomly through the ICSI or IVF procedure. Embryo quality was examined.
Results: The mean age, basal follicle-stimulating hormone (FSH), and number of oocytes recovered for the patients were all comparable between the two study groups. The total number of blastocysts of oocytes cultured with and without CCs by ICSI procedure was significantly higher than IVF technique (57 vs. 16; P=0.000).
Conclusion: Findings of the current study revealed that the embryo quality of in vitro matured oocytes during ICSI procedure is higher than IVF method.
Keywords: Fertilization, Cumulus Cells, Immature Oocytes, Intracytoplasmic Sperm Injection

P-104: A Study of Couple Burnout in Infertile Couples

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Background: Infertility is a major crisis that can cause psychological problems and emotionally distressing experiences, and eventually affect a couples' relationship. The objective of this study is to investigate couple burnout in infertile couples who were undergoing treatment at the Infertility Clinic of Yazd, Iran.
Materials and Methods: The present study is a cross-sectional descriptive one on 98 infertile couples referring to the Infertility Center of Yazd, Iran, who were chosen on a simple random sampling basis. The measuring tools consisted of the Couple Burnout Measure (CBM) and a demographic questionnaire. The collected data were analyzed using SPSS 16 and the statistical tests of ANOVA and t test. P values less than 0.05 were considered as significant.
Results: The results show that infertile women experience higher levels of couple burnout than their husbands (P<0.001). Also, a comparison of the scales of couple burnout psychological burnout (P<0.01), somatic burnout (P<0.01), and emotional burnout (P<0.001) between wives and husbands show that women are at greater risk.
Conclusion: Infertile couples' emotional, mental, and sexual problems need to be addressed as part of the infertility treatment programs, and psychotherapists should be included in the medical team.
Keywords: Burnout, Couples, Infertility

P-105: Violence and Sexual Function between Fertile and Infertile Women

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Background: Fertility is one of the most important variables. Infertility and others' attitudes towards this factor make infertile couples vulnerable to mental and emotional disturbances, which ultimately lead to sexual dysfunction and domestic violence. The present study aimed to investigate violence and sexual function among fertile and infertile women.
Materials and Methods: This was a comparative study on 294 individuals (147 infertile women and 147 fertile women) visiting women's clinic in Jahrom city from April to October in 2017. The research tools were Domestic Violence Inventory and Female Sexual Function Index (FSFI). T test was used to compare means between the groups and chi-squire was used to investigate the relationship of domestic violence with other variables.
Results: Comparison of dimensions of domestic violence between fertile and infertile showed that physical violence (P=0.01), sexual violence (P=0.02) and psychological violence (P<0.0001) were higher in infertile women than fertile women and this increase was statistically significant. Comparison of sexual function dimensions between the two groups showed that all dimensions were significantly lower in infertile women than fertile women (P<0.05). No significant relationship was also found between domestic violence and sexual function in infertile women.
Conclusion: The results showed that dimensions of violence were higher in infertile women than fertile women and sexual function was lower in infertile women than fertile women. Therefore, health managers and politicians should pay specific attention to infertile women and include psychological and sexual counseling alongside infertility treatments. On the other hand, screen for domestic violence should be practiced in infertile women as high-risk group.
Keywords: Infertility, Violence, Sexual Relation

P-106: Infertility and Confusion in The Choice of Childbearing Methods in A Sample of Iranian Women with Premature Ovarian Insufficiency: A Qualitative Study

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Background: Premature ovarian insufficiency (POI) is a condition associated with female infertility that affects approximately 3.7% of women under the age of 40. For the POI patient, this is one of the most dramatic problems. The chance for spontaneous conception is very limited and ranges from 4 to 8%. For contemporary medicine, infertility treatment in POI patients is a challenge. At present, oocyte donation is regarded as the only proven method in the treatment of infertility in POI patients. However, nowadays we can observe important progress in the development of fertility preservation methods such as cryopreservation of oocytes, embryos, and ovarian tissue. Additionally, new methods known as in vitro activation of dormant follicles and possible use of stem cells should be mentioned. Nevertheless, some of couples are looking for quick solutions to have children and are preferring to have adopted children. The present study aimed to explain the couple's confusion in choosing the method of childbearing.
Materials and Methods: This was a qualitative study using in-depth semi-structured interviews with 16 women with POI selected via purposive sampling method. The data from interviews were then analyzed using content analysis method.
Results: Three subcategories emerged for confusion in the choice of childbearing methods: rejection of adopted children (rejection of the adopted child by the couple or the spouse's family), Hard acceptance of donated eggs (feeling compelled to accept a donated egg, shocked and reluctant to accept a donated egg, spouse's opposition to accepting a donated egg), the importance of other aspects of donated egg (the importance of the religious aspects of the donated egg, the desire to identify the donor, the importance of the donor being familiar).
Conclusion: There is a strong need to provide counseling services to couples to introduce various methods of childbearing as well as clarifying each method by providing explanations will reduce their confusion and uncertainty.
Keywords: Childbearing, Infertility, Primary Ovarian Insufficiency, Qualitative Evaluation

P-107: Potential Therapeutic Effect of Bee Pollen and Metformin Combination on Testosterone and Estradiol levels, Apoptotic Markers and Total Antioxidant Capacity in Rats model of Polycystic Ovary Syndrome

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Background: Polycystic ovarian syndrome (PCOS) is associated with metabolic syndrome as well as infertility. Many traditional remedies have been reported to show estrogenic and antioxidant potential. Bee pollen (BP) is a natural compound, reported as one such remedy. The present study aimed to investigate the effects of Bee Pollen extract (BPE) and Metformin (MET) on Estradiol (E2) and testosterone levels, apoptotic markers, and total antioxidant capacity in rat's model of polycystic ovary syndrome.
Materials and Methods: In this experimental study, 54 Wistar (n=6/group) female rats have injected 2 mg of estradiol valerate, (EV) intramuscularly and 6 rats were considered as the control without EV injection. The rats were treated with BPE (50, 100, 200 mg/kg) and MET (300 mg/kg), either individually or in combination. Serum levels of E2 and testosterone were assessed by ELISA method. The total antioxidant capacity (TAC) of serum was also determined. The expressions of Bcl2, Bax, Caspase 3, and SIRT1, genes were evaluated by real-time PCR. Data were statistically analyzed using one-way ANOVA.
Results: In PCOS group E2 and testosterone levels ($P<0.01$), and Bcl2 ($P=0.007$) expression were increased, but TAC ($P=0.002$) and expression of Bax ($P=0.001$), Caspase 3 ($P<0.01$) and Sirt1 ($P<0.01$) were decreased significantly. The levels of E2 and testosterone, as well as the expressions of Bcl2, were decreased in the treated groups compared to the PCOS group ($P<0.01$). On the other hand, TAC and expression of Bax, Caspase 3 and Sirt1 were increased in the groups treated with BP and MET compared to the untreated group ($P<0.05$).
Conclusion: Bee pollen synergistically with metformin, improved serum E2, TAC, and testosterone, and expression of apoptotic genes.
Keywords: Polycystic Ovarian Syndrome, Total Antioxidant Capacity, Bee Pollen, Metformin, Estradiol

P-108: Level of Zinc in Follicular Fluid, Oocyte Morphology and Embryo Quality in Women with Polycystic Ovary Syndrome Undergoing *In Vitro* Fertilization Treatment

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Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. Zinc is a vital trace element in the body that plays an important role in health, especially due to its antioxidant role. On the other hand, lack of antioxidants and the existence of oxidative stress can adversely affect oocytes quality and consequently fertility rate. The effects of follicular fluid zinc on the number and quality of the oocytes in infertile women with PCOS have not been studied so far. We decided to investigate this issue.
Materials and Methods: This is a cross-sectional study which was conducted on follicular fluid samples, oocytes, and embryos collected from 90 PCOS women undergoing in vitro fertilization in the Omolbanin Hospital, Dezful, Iran, from February to December 2019. Their zinc level was measured using HPLC. Oocytes morphology and embryos quality were evaluated using inverted optical microscopy.
Results: The amount of the follicular fluid zinc did not cause

any significant difference in the number of oocytes and metaphase I (MI) and germinal vesicle (GV) oocytes, but there was a significant decrease in the number of metaphase II (MII) oocytes at zinc values less than 35 µg/dL. The follicular fluid zinc levels less than 35 also decreased the embryo quality significantly.
Conclusion: There was a significant relationship between the level of follicular fluid zinc and the quality and the number of oocytes taken from the ovaries of infertile patients with PCO history who were candidates for in vitro fertilization treatment as well as the number of more high quality embryos.
Keywords: Polycystic Ovary Syndrome, Zinc, Oocyte, Embryo

P-109: Effect of Weight Loss on Health-Related Quality of Life in Overweight Women with Polycystic Ovary Syndrome (PCOS)

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Background: Excess body weight are present in 30-70 % of PCOS women and both situation may affect HRQOL. Weight loss is the first step in treatment of polycystic ovary syndrome (PCOS) in overweight women. The aim of this study is to evaluate the effect of weight loss on HRQOL.
Materials and Methods: Participants aged 18-40 years old with body mass index (BMI)>25 and ≤38 kg/m² were invited to participate in the study and followed a 24-week dietary intervention. The diagnosis of PCOS was based on Rotterdam criteria. Out of 98 PCOS women who were included, 65 participants completed the study. Energy restricted Low glycemic diet was prescribed according to subject's BMI of 22 kg/m² and a deficit of 500 Kcal per day that produce 0.5 kg weight loss per week. All participants were asked to maintain their baseline physical activity. BMI was assessed at week 0, 12, 24 of intervention. The validated PCOS questionnaire (PCOSQ) was measured at week 0, and 24 weeks after intervention.
Results: Compared to baseline, after 24-weeks of intervention, a significant decrease in BMI was detected (8.5% ± 3.5 P<0.001). Menstrual regularity was retained in 67% of PCOS women with irregular menses. By week 24 significant improvement in PCOSQ scores were reported. There was a significant correlation between percent of weight loss and total PCOSQ score (r= -0.7, P<0.001), the emotion domain (r= -0.6, P<0.001), weight domain (r= -0.75, P<0.001) menstrual domain (r= -0.58, P<0.001), and infertility domain (r= -0.4, P<0.001). The correlation between percent of weight loss and body hair domain score was not significant (r= -0.27, P=0.1).
Conclusion: In summary, this study shows that weight loss in overweight PCOS women can improve some aspects of HRQOL.
Keywords: Polycystic Ovary Syndrome, Weight Loss, HRQOL

P-110: Metformin Attenuates Angiogenic and Inflammatory Genes Expression and Proliferation in Human Endometriotic Stromal Cells

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Background: Endometriosis is a benign gynecological disease that is manifested by the presence and growth of endometrial cells and glands outside the uterine. Active angiogenesis, migration, and invasion of endometrial tissue outside the uterine seem to be vital for the development of endometriosis and lead to the survival and growth of endometriotic lesions. Metformin, as an anti-diabetic agent, has shown anti-angiogenic properties. Here, we performed a study using human normal endometrial stromal cells (N-ESCs) from healthy endometrial tissue and human eutopic endometrial stromal cells (EU-ESCs) and ectopic endometrial stromal cells (ECT-ESCs) from endometriosis patients.
Materials and Methods: N-ESCs biopsies were obtained from women undergoing benign surgery and ECT-ESCs and EUO-ESCs were obtained from endometriosis patients undergoing diagnostic laparoscopy. ESCs were cultured and treated with different concentrations of Metformin (0-20 mmol/l) for 72 hours to evaluate the impacts of Metformin on the expression of angiogenesis and migration markers. The Metformin effect on cell viability, proliferation, migration was measured by MTT assay and scratch test.
Results: Immunofluorescence staining of the ESCs showed positive results for Vimentin, a stromal cell cytoskeletal marker and negative signal for Cytokeratin, an epithelial marker. The flow cytometric data showed that ESCs were positive for CD29, CD73, and CD90 (mesenchymal origin antigens) but were negative for CD31 and CD45. The proliferation and viability of ESCs were affected by Metformin in a time and dose-dependent manner. Metformin could significantly alter the expression of VEGF-A, MIF, MMP-2, MMP-9, TIMP, HIF-1α genes which are critical for angiogenesis and cell migration in ESCs (P<0.05).
Conclusion: Our data showed anti-angiogenic properties of Metformin on ESCs from endometriosis patients. Metformin attenuates angiogenic and inflammatory gene expression and migration of endometriotic cells. Our findings suggest that Metformin may be useful as an adjunct to current endometriosis treatments.
Keywords: Endometriosis, Metformin, Infertility

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 intraperitioneal 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 fac-

tor 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 Microsurgical 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

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

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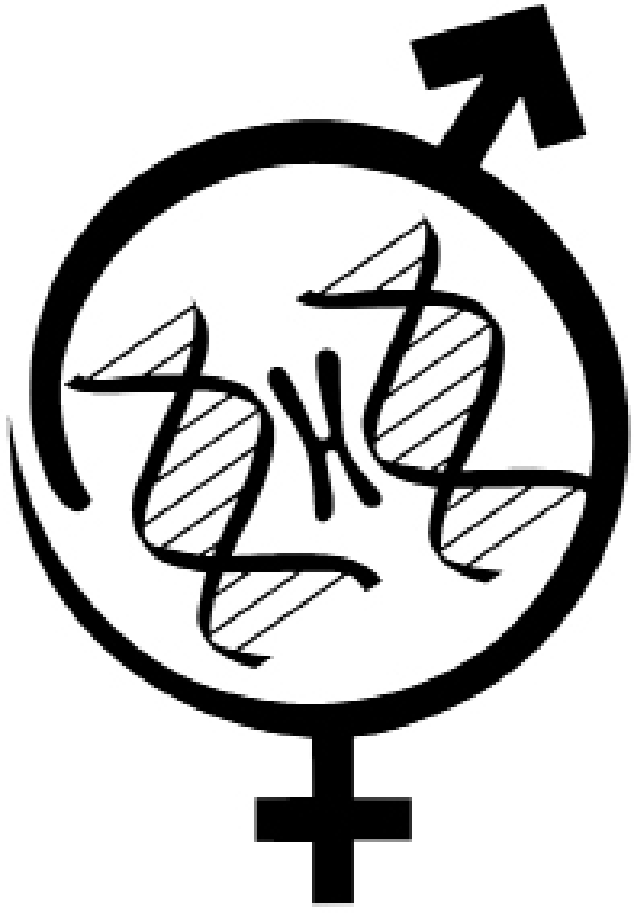

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Inm-1: The Role of Nurses and Midwives in POF Counseling and Treatments

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

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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-3: The Influence of Infertility on Various Domains of Women's Lives

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Background: Childbearing as an important concept of marriage can be affected by infertility and tubal ligation (TL). In this study, an attempt was made to evaluate anxiety, depression, body image, self-esteem, sexual function (SF), and quality of life (QoL) in Iranian infertile women and to compare the results with fertile women and those with TL.
Materials and Methods: In this cross-sectional study, a total of 600 women who had; infertility (study group; n=200), undergone TL (control 1; n=200) and used a condom (control 2; n=200) were enrolled between 2017 and 2019 at Royan institute and health care centers in Tehran (Iran). They were required to fill out the Short Form Health Survey (SF-12), Female Sexual

Function Index (FSFI), Hospital Anxiety and Depression Scale (HADS), Body Image Concern Inventory (BICI), and Rosenberg' Self-Esteem Scale (RSES). One-way ANOVA was used to test differences between groups.
Results: All mean values were found to be lower in the TL women and the differences between the three groups were statistically significant in all domains. Women with TL had more female sexual dysfunction (22.43 ± 5.30 vs 24.79 ± 4.74 vs 28.03 ± 3.29, P<0.001). There was a significant difference between the three groups in SF-12 scores (76.59 ± 13.14 vs 68.49 ± 14.47 vs 78.87 ± 12.62, P<0.001). Also, there was a significant difference between the three groups in anxiety, depression and total scores HADS (P<0.001). Infertile women had lower body image (P<0.05). Self-esteem was lower in TL groups (P<0.05).
Conclusion: Our findings reflect the potential adverse effects of TL on the anxiety and depression, sexual life, body image, and QoL of women. It is suggested that the awareness and knowledge of health-care professionals regarding the effects of TL on all aspects of women's life can play a critical role in women's health.
Keywords: Infertility, Tubal Ligation, Sexual Function, Quality of Life, Depression

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 composed 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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