

خلاصات  
رسائل الماجستير  
في  
علم الاجنة التطبيقي

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في فسلجة التناسل السريري

الباحثة : أسراء محسن عبد الهادي

بإشراف : الاستاذة الدكتورة سعيدة علي الانصاري

التاريخ : 2003

*The effect of caffeine on human sperm activation and sperm penetration tate of asthenospermic patients*

## SUMMARY

*The effect of in vitro addition of caffeine to semen of 118 infertile patients complaining from asthenospermia was studied in the present research work. The objective of the study was to use caffeine for in vitro sperm activation of asthenospermic semen of infertile patients. The effect of caffeine on human sperm penetration percentage was also studied.*

*Two in vitro sperm activation techniques were used (Direct and indirect techniques) and three levels of caffeine concentrations (1mM, 3mM and 6mM) were applied in this work. Sperm penetration assay (SPA) using zona free hamsters oocytes was performed to determine human sperm fertilizability rate in the presence or absence of 3mM caffeine supplementation to the sperm preparation medium. The data showed that caffeine supplementation (1mM, 3mM and 6mM) in a direct method resulted in a significant increase ( $p<0.001$ ) in sperm function tests (sperm motility percent, grade activity and sperm motility index) compared to the control group. Best improvements were observed in 3mM caffeine concentration. Sperm function tests (sperm concentration, sperm motility percent, grade activity, normal morphology percent and sperm motility index) were significantly enhanced when 3mM caffeine concentration was added to the sperm preparation medium in the indirect method compared to the control group.*

*The in vitro human sperm penetration rate and penetration index were significantly increased ( $p<0.001$  and  $p<0.01$  respectively) in the caffeine treated group when compared to the control group. Caffeine found to increase sperm motility and fertilizability in asthenospermic infertile patients when applied to in vitro sperm*

*activation. It was concluded from the results of this work that the application of 3mM caffeine to in vitro sperm activation may be useful for intrauterine insemination and in vitro fertilization, to achieve high pregnancy rate .*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث: رشا نوري عبد مطرود

بإشراف : الاستاذ الدكتور سعد صالح الدجيلي

التاريخ : 2007

*Effect of certain additives to tris solution for maintenance of human semen during cryostorage*

## SUMMARY

*The aim of this study is to investigate the effect of modified Tris solution (MTS) mixed with Glycyrrhiza glabra extract (Gg) and egg yolk on maintenance of human semen several days following ejaculation to be used for repeated intra- uterine insemination (IUI). This study was conducted in the laboratories of the Institute of Embryo Research and Infertility Treatment, AL-Nahrain University through the period from June 1-7-2006 to March 1-3-2007. One hundred and twelve semen samples were included in this investigation. The men ages ranged between 25-45 years. The study included four experiments:*

*Experiment No.1: The efficiency of MTS (Tris+fructose +citric acid) mixed with 20% Gg and 30% egg yolk for cryostorage of washed and un-washed semen for different periods 24, 48 and 72 hrs and then activated in vitro on certain human sperm function parameters namely; sperm concentration, active sperm motility grades A and B, and the percentage of morphologically normal sperm (MNS) of infertile men.*

*Experiment No.2: Comparison of certain sperm function parameters between fertile and infertile men following in vitro activation of washed and un-washed cryostorage semen for 24, 48, 72 hrs using MTS mixed with 20%Gg and 30% egg yolk .*

*Experiment No.3: Comparison between the effect of adding 10% egg yolk and 30% egg yolk to MTS mixed with 20% Gg for cryostorage of washed and un-washed semen on certain human's sperm function parameters following in vitro activation technique.*

*Experiment No.4: Comparison between the effects of adding 20%Gg and free 0% Gg to modified Tris solution mixed with 30% egg yolk for cryostorage of washed and un-washed semen on certain human's sperm function parameters following in vitro activation technique.*

*The results showed a significant ( $P<0.05$ ) effect of MTS mixed with 20% Gg and 30% egg yolk on certain human sperm function parameters that activated in vitro following different periods of cryostorage as compared with before activation. There was a significant ( $P<0.05$ ) increase in sperm concentration, active sperm motility grade A and the percentage of MNS of fertile semen compared with infertile semen following cryostorage process of washed and un-washed semen for 24, 48 and 72 hrs. Adding 30 % egg yolk to modified culture media for cryostorage process for 48hrs and in vitro activation have a significant ( $P<0.05$ ) improvement of active sperm motility grade A (30.40%) compared with adding 10% egg yolk grade A (16.5%). Cryostorage process with MTS and 20% Gg showed a significant ( $P<0.05$ ) elevation in sperm concentration (50.8 m/ml) active sperm motility grade A (33.70%) and grade B (42.20%) compared with using MTS mixed with 0% Gg sperm concentration (45.9 m/ml) grade A(23.80%), and grade B(36.00%) following in vitro activation technique .It is concluded that MTS mixed with 20% Gg and 30% egg yolk is suitable for cryostorage of human sperm and in vitro activation following several days of ejaculation .Thus, from these results can be utilize preserved spermatozoa for repeated IUI.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : باسم خميس كوتي

بإشراف : الأستاذ المساعد الدكتور محمد باقر محمد رشاد

التاريخ : 2007

*An evaluation of hypo-osmotic swelling test regarding: Seminal fluid analysis, sperm preparation techniques, and outcomes of intra-uterine insemination*

## SUMMARY

*The hypo-osmotic swelling (HOS) test introduced as a clinical, physiological, and non deleterious test. However, it may be used as an optional, additional, and viability test. In addition, it is easy to score and give additional information on the functional integrity of plasma membrane and fertilization potential of human spermatozoa. Actually, the functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction (AR), and the binding of the spermatozoa to the egg surface. Therefore, the present study focused on usefulness of the HOS-test in predicting pregnancy rate in couples undergoing intrauterine insemination. In addition to study the effect of SFA parameters, in vitro sperm activation techniques, and sperm stimulators on human sperm HOS-test were assessed. One hundred infertile couples with male infertility factors were involved in this study during their attendance at Institute for Embryo Research and Infertility Treatment/Al-Nahrain University. From each infertile male, semen sample was taken and assessed the parameters of SFA. Moreover, in vitro sperm activation was performed using either direct swim-up or centrifugation swim-up techniques with or without presence of stimulators. Then, intrauterine insemination was done after sperm activation and processing. The effect of age, duration of infertility, smoking habit on sperm functions and sperm HOS-test were studied. Crude data were statistically analyzed. The mean age for infertile subjects in this study was  $(31.35 \pm$*

*0.66) years and duration of infertility 20(16.66 ± 0.33) years. The number of infertile patients with primary infertility was four times higher than the number of infertile patients with secondary infertility. The mean ages of primary and secondary*

*infertility were (31.01±0.74) and (32.75±1.53) years; respectively. All semen parameters of infertile men were significantly ( $P<0.001$ ) improved after in vitro sperm activation and preparation with and without addition of (0.5mg/ml) Pentoxifylline or (5%) human serum albumin. direct swim-up technique gave results for sperm activation and IUI better than the centrifugation swim-up techniques. Additionally, it was observed that the best improvement in parameters of SFA, sperm HOS-test, and outcome of IUI for non smokers rather than smokers. The scores of HOS-test were impaired in semen samples of infertile patients with advanced age, duration of infertility, smoking habits, and in oligoasthenozoospermic patients with varicocele. In the present study, eighteen pregnancies were reported using sperm preparation technique and intrauterine insemination. Actually, according to type of infertility, thirteen pregnancies for patients with secondary infertility and five pregnancies for patients with primary infertility were assessed. Moreover, all pregnancies (No. =18) were achieved for infertile couples have normal HOS-test scores according to criteria of WHO (1999). Eventually, it was concluded from the results of this study that sperm HOS-test were positively correlated with the fertilization potential of human spermatozoa and give optimistic predictive value for pregnancy rate for infertile couples undergoing intrauterine insemination. Ultimately, the HOS-test is simple, useful, valuable, accurate, reproducible, reliable, diagnostic, and prognostic tool for fertilization potential of human spermatozoa and ongoing pregnancy rates.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : مفيدة علي جواد عليوي

بإشراف : الأستاذ المساعد الدكتور محمد باقر محمد رشاد

التاريخ : 2008

*Assessment of changes in reproductive hormones and clomiphene citrate challenge test for evaluation of the outcome of intrauterine insemination of infertile females*

## SUMMARY

*The human's ovarian function declines with life, so the pregnancy rates decrease progressively with an increase of miscarriage rates. Therefore, the present study was focused on assessment of ovarian function by different parameters. One hundred fifty infertile couples were involved in this study during their attendance at Institute of Embryo Research and Infertility Treatment /Al-Nahrain University during the period from August 2006 to August 2007. The mean age for infertile females in this study was  $(35.14 \pm 0.67)$  years old. They were assessed in the first cycle for basal FSH, LH and E2. Clomiphene citrate was given for all females in a dose of 100 mg/day for five days from cycle day five to cycle day nine, FSH was re-examined in cycle day ten of the first cycle. Four females were excluded from the study because they became pregnant on clomiphene citrate only in the first cycle. In the next cycle recombinant FSH 75 IU was given for all females (146) in an adjusted doses, then an intrauterine insemination was done for all females who had dominant follicle (18-23mm) demonstrated by vaginal ultrasound. IUI results were assessed as a cycle cancellation rate and pregnancy rate according to age, duration of infertility, type of infertility, basal (FSH, LH, E2), FSH: LH ratio, LH: FSH ratio, endometrial thickness, number of Graffian follicles and cycle day ten FSH after CCCT, then these parameters were compared with each other according to the highest cycle cancellation rate from doing IUI and it was found that the highest cycle cancellation rate was detected by using CCCT (92.5%), and the highest IUI*



*rate was detected by using the same test (78.7%). While the weakest test used for detection of cancellation rate and IUI rate was by using cycle day 3 LH*

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معهد أبحاث الأجنة وعلاجات العقم

رسالة الماجستير في علم الأجنة التطبيقي

الباحث : علاء شعلان حاجم سلطان

بإشراف : الاستاذ الدكتور سعد صالح الدجيلي

الاستاذ المساعد الدكتور انتصار نعمان العزاوي

التاريخ : 2008

*The effect of Glycyrrhiza glabra on the in vitro fertilization in mice.*

## SUMMARY

*The objective of the present study was to investigate the possibility of using Glycyrrhiza glabra (G.g) for in vitro sperm direct activation technique ,in vitro fertilization and early embryonic development of mice. Glycyrrhiza glabra 10% of HTF culture medium was used with in vitro direct sperm activation technique for one hour incubation period before insemination. The same concentration was also used for the inseminated oocytes and for the cultured embryos after 24 hours of insemination. The early developed embryos were re-cultured with G.g 10% of KSOM-AA medium for 48 hours after insemination. According to the time of in vitro fertilization, the superovulated female mice were divided into two groups: one group with 367 oocytes were collected and inseminated with G.g-free HTF medium (the control group), and the second group with 373 oocytes were collected and inseminated with 10% G.g-HTF medium (the treated group). Each group was inseminated with the same sperm concentration ( $1 \times 10^6$  sperm/ml). The fertilization rate was recorded after 24 of insemination, while embryonic development rates were recorded after 24 and 48 hours of insemination. The results indicated that addition of G.g with the direct activation technique showed positive effects on epididymal sperm concentration, sperm motility percent and grade of progressive forward movement. There was a highly significant ( $P < 0.001$ ) increase in fertilization rate of*

*the treated group with 10% G.g-HTF (53.89%) compared to G.g-free HTF medium (36.82%) after 24 hours of insemination. Embryonic developmental rate was significantly increased after 24 and 48 hours of insemination. The study showed that the quantity and quality of embryos generated from the treated group were*

*superior to that of untreated group in embryonic development and in embryo grades. IX The results were attributed to the effect of various components of G.g, especially the carbohydrates, amino acids, antioxidants compounds, vitamins and estrogenic and anti estrogenic substances with various trace elements. It is concluded from the present study that the addition of G.g powder extract to the culture media of sperm and oocyte can enhance the fertilization rate, embryonic development and embryo quality in mice. This result can be used for other mammalian IVF programs.*

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معهد أبحاث الاجنة وعلاج العقم  
رسالة الماجستير في علم الاجنة التطبيقي  
الباحث: ضحى سالم نعمة حسن  
بإشراف :الأستاذ الدكتور اسامة سليمان الناصري  
الأستاذ الدكتور حذامة رزوقي حسن  
التاريخ : 2008

*Effect of vitamin E and zinc treatment on oxidative stress in some Iraqi infertility .*

## SUMMARY

*The goal of this study was to elucidate the interrelations between oxidative stress (O.S) in seminal fluid and antioxidant inducing disruption of spermatogenesis. Forty six infertile patients aged (24-41) years were involved in this study. These infertile patients group were divided into two groups: the first group (Zinc group n=22), who were received zinc treatment and the second group (vit E group n =24) who were received vitamin E ( $\alpha$ - tocopherol). Each group (zinc group & vit E group) were sub classified according to the WHO criteria (1999) into three different subgroups: oilgoasthenoteratozoospermia (OAT) Asthenoteratozoo- spermia (A.T), and Asthenozoospermia (Asthen.).The control group consist of samples obtained from healthy donors of proven fertility (n=23). Six main parameters were determined in seminal fluid of infertile patients and healthy men. These are the sperm and seminal plasma Malondialdehyde (MDA) concentration, athiobarbituric acid-reactive substance (TBARS), and measurement of superoxide dismutase (SOD) activity, total scavenging activity, zinc concentration and vitamin E concentration in seminal plasma. The results showed that, there was a high significant decrease ( $P \leq 0.001$ ) in sperm parameters (concentration, progressive motility and normal morphology), SOD activity, total scavenging activity, zinc concentration and vitamin E concentration in infertile group compared with that fertile group .Moreover, analyzing the statistically difference in the different*

*measured parameters between the zinc group after treatment in comparison with that before treatment was observed the following:*

- 1 - A high significant increase ( $P \leq 0.001$ ) in seminal fluid parameters including progressive motility and normal morphology, with a significant increase ( $P \leq 0.05$ ) in the sperm concentration in zinc group.*
- 2- A high significant decrease ( $P \leq 0.001$ ) were found in total [MDA] and a significant decrease ( $P \leq 0.05$ ) in sperm [MDA] (MDAp), whereas, no significant difference ( $P > 0.05$ ) in the seminal plasma [MDA] (MDAs).*
- 3 - A high significant increase ( $P \leq 0.001$ ) were found in seminal plasma total scavenging activity and zinc concentration, whereas no significant difference ( $P > 0.05$ ) was observed in the seminal plasma SOD activity.*
- After analyzing the statistically difference in the different measured parameters between the vit E group after treatment, in comparison with that of before treatment; the following was observed:*
  - 1- A high significant increase ( $P \leq 0.001$ ) was found in progressive motility and a significant increase ( $P \leq 0.05$ ) in the sperm concentration. Whereas, no significant difference ( $P > 0.05$ ) was observed in normal morphology.*
  - 2- A high significant decrease ( $P \leq 0.001$ ) were found in the sperm [MDA] (MDAp), with a significant decrease ( $P \leq 0.05$ ) in total [MDA] and in the seminal plasma [MDA] (MDAs).*
  - 3- A high significant increase ( $P \leq 0.001$ ) were found in seminal plasma total scavenging activity and vitamin E concentration, whereas no significant difference ( $P > 0.05$ ) was observed in seminal plasma SOD activity.*

*From all of these observations, it can be concluded that O.S contributes most likely to sperm damage and may be responsible for poor seminal fluid characteristic of the infertile patients involved in the present study. Antioxidants (zinc & vitamin E) play an important role in the improvement of seminal fluid parameters.*

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معهد أبحاث الاجنة وعلاج العقم  
رسالة الماجستير في علم الاجنة التطبيقي  
الباحث : نسرین خزل فليح محمد  
بإشراف : الاستاذة باسمه محمد الجبوري  
التاريخ : 2008

*Effect of licorice ( Glycyrrhiza glabra) administration on the pregnancy outcome and birth rate in the famale mice*

## SUMMARY

*This study aims to assess the effect of the Glycyrrhiza glabra extract consummation by the mature female mice on the fate of pregnancy, birth rate, and offspring wellbeing. Sixty female mice of age of six weeks are used as a model for this study.*

*The female were divided into three experimental groups (15 mice/ group) parallel with three control groups (5 mice/ group). The first experimental group (G1) administrated with 1gm/ kg b.wt of Glycyrrhiza glabra extract dissolved in 1 mLof distilled water orally per day for three weeks before mating while the control group is given the same quantity of distilled water only for the same period. The second experimental group (G2) is given 1gm/kg b.wt. Of Glycyrrhiza glabra.extract in the same previous method for 6 weeks, i.e. the administration continues during pregnancy period while the corresponding control animal receives distilled water only for the same period. The third experimental group (G3) administrated the same dose of Glycyrrhiza glabra extract with the same route followed in G1 and G2 but during the pregnancy period only (three weeks after mating).*

*At the beginning of the experiment, vaginal smear is taken from all animals daily until metestrous phase and mating occur and the first day of pregnancy registered. The registration of pregnancy duration, birth number, birth weights in the first day of birth and re-checking of births weights and its reproductive organs*

*in mature age (5weeks) are done .After three weeks of delivery the mothers are killed and the assessment of follicle-stimulating hormone (FSH),*

*Luteinizing hormone (LH), and estradiol hormone (E2) is done, and also, the weights of the mothers reproductive organs are measuredThe data reveal a significant reduction ( $P<0.05$ ) in the length of pregnancy duration and a highly significant increase ( $P<0.01$ ) in births number in all experimental groups compared to the control group. Also, a significant increase( $P<0.01$ ) occurs in pups' weights and their reproductive organs weights, in addition to the mothers' reproductive organs weights treated with Glycyrrhiza glabra extract in comparison with their control groups. The hormonal assessment shows a highly significant increase ( $P<0.01$ ) in E2 level and a significant increase ( $P<0.05$ ) in FSH and LH levels of all mothers in experimental groups compared to that of the control groups.*

*The difference in the dosing administration period of Glycyrrhiza glabra extract between the three experimental group G1, G2, G3 (3weeks before pregnancy and 6 weeks before and during pregnancy and 3weeks during pregnancy) reveals a more positive effect when the Glycyrrhiza glabra extract administrated for longer period which is noticed in a higher rates in births weights, and hormones of G2 compared to G1 and G3. This study concludes that the daily oral use of Glycyrrhiza glabra extract in a small dose (0.003 mg) leads to precocious delivery with an increase in the pups numbers without any side effects on weights or normality of both the pups and mothers. These results indicate the importance of this herb in the enhancement of fertility, pregnancy, and birth rates.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : عايدة حميد حسن جعفر

بإشراف : الأستاذ الدكتور أنعم رشيد الصالحي

الأستاذ الدكتور كوكب سليم نجم

التاريخ : 2008

*Morphological and morphometric study of the perinatal development of rat renal glomerulus*

## SUMMARY

*This study is concerned with the perinatal development of the kidney. It concentrates on the glomerular development in rat during the later intrauterine life and early post natal days, morphologically by using the light microscope and electron microscope, and by the morphometric evaluation of the filtration slit diameter during this period and then correlating the morphological and morphometric changes with the functional maturation of the glomerulus. The work was performed on rat (*Rattus rattus norvegicus albinus*). It covered the period of preterm days before birth and the first 8 post natal days. Sections of the kidney in the different ages of the study period were stained by H&E for the paraffin section. The methylene blue for the semi thin plastic section for the light microscope and the thin plastic section stained with uranylacetate and lead citrate for electron microscope. BEL image analyses system was used to measure the diameters of the filtration slit. This study showed that the formation of the glomerulus continues during the first few days after birth and by the end of the first week, the cortical region becomes identical to mature kidney. The embryonic development of the kidney dose not complete during the intrauterine life. The neonatal kidney shows well defined standard and sequential morphological stages. It is morphologically distinct from that in adults. Each glomerulus passes though stages which include:*



*vesicle stage, comma body shape stage, "S" shaped stage, double comma stage, elongation stage and ovale stage that ends by the mature glomerulus. The*

*formation of the filtration barrier components is complete at the ovale stage and spatial relationship between the components of the filtration barrier in the kidney is well establish at birth, with well defined morphological features .The diameter of the filtration slit at birth is  $(12.2368 \pm 1.7 \text{ nm})$  that significantly increases until reaching to  $(25.3991 \pm 4.3 \text{ nm})$  by the end of the first week. The changes in the filtration slit diameters affecting the filtration coefficient (Kf) are proposed to be the cause behind the low glomerular filtration rate in the early post natal kidney.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : لقمان جمعة توفيق

بإشراف : الأستاذ الدكتور محيسن حسن عداي

الأستاذ الدكتور وليد حميد يوسف

التاريخ : 2008

### *Effect of tribulus terrestris extract on reproduction in mature male mice*

## SUMMARY

*This study was conducted to investigate the effect of Tribulus terrestris (T. terrestris) extract on the reproductive system and hormonal status of mature male mice. The animals were divided into four groups (15 mice for each group). First group (control) treated with distilled water orally every day. The second, third and fourth groups were administered aqueous extract of T. terrestris at doses (75, 150, 300mg/kg/day) respectively. After two weeks of experiment 5 mice were weighed from each group and sacrificed to get the weight of reproductive organs (testis, epididymis and seminal vesicles). Histological sections were done and the level of FSH, LH and testosterone were measured. The epididymis was minced for extraction of the sperm from it to find the effect of the plant on it. At the fourth week of treatment the same works were done on other 5 mice from each group and the remaining 5 mice from each group were mated with untreated (9 – 12 weeks old) female mice (each male with three female mice) for determination of the fertility capacity. After two weeks of treatment, the results showed a significant increase in serum FSH, LH, and testosterone concentrations. A remarkable increase was noticed in body, testis, seminal vesicles and epididymis weights. Sperm concentration, motility percent and grade of motility showed a significant increase in treated groups. The histological sections showed a significant increase in the diameter and height of the epithelial lining of the seminiferous tubules and epididymis. At the fourth week of treatment, the above*

*mentioned parameters showed a similar trend. The results also showed an increase in the number of fetuses of treated groups when compared with the control group. The results of the present study demonstrated the positive effects of T. terrestris extract on reproductive performance of male mice.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : ليلى عبد الرازق خميس جودة

بإشراف : الاستاذ المساعد الدكتور محمد باقر محمد رشاد

التاريخ : 2008

*Human sperm survival assay as a toxicity test in assisted reproductive technology laboratory*

# SUMMARY

*Despite advances in assisted reproduction, there is no progress in quality control (QC) bioassays. The role of QC procedures in the in vitro fertilization (IVF) laboratory, is to fine-tune existing protocols in order to more effectively help infertile patients in their quest to have a healthy baby. The human sperm survival assay (HSSA) was used as a proficient measure of QC in the assisted reproductive technology (ART) laboratories. This assay provides a reliable, economical, rapid, and easy performed quality control method. Also this test minimizing killing of laboratory animals, like mice, rats, and rabbits, and use of human cells instead.*

*In the present study, we have investigated the toxic effects of several biochemicals, chemicals, and other materials commonly used in ART laboratories, by examining its effects on human sperm motility, progressive motility, viability, survival index (SI), and toxicity index (TI). One hundred and eight semen samples with normal parameters collected from healthy men during their attendance at The Institute of Embryo Research and Infertility Treatment / Al-Nahrain University, were involved in this study, which have been extended from August 2006 to August 2007. Items tested in this study included two concentrations of each of the following: human serum albumin (HSA; 10% and 5%), bovine serum albumin (BSA; 10% and 5%), sucrose (0.2 M and 0.1 M), and mannitol (0.2 M and 0.1 M), two types of water used for culture medium preparation (Milli-Q high purified water; MQW and distilled water; DW), two types of gloves (sterile and non sterile), two types of catheters*

*(intrauterine insemination; IUI-catheter), and embryo transfer; ET-catheter), and two volumes of alcohol (ethanol; 0.2 mL and 0.1 mL). HSSA was conducted after 0, 1, 2, and 24 hrs incubation intervals under standard embryonic*

*conditions, where sperm parameters examined. A calculated sperm SI value  $< 0.75$  was used to indicate sperm toxicity.*

*Our results showed that each of the two concentrations of both HSA and BSA did not show any toxic effect through 2 hrs period of sperm incubation, but 5% HSA caused toxicity at 24 hrs time. In addition, the two concentrations of sucrose were toxic at 24 hrs time. Another carbohydrate, mannitol at its two concentrations examined in this work exhibit toxicity at 2 hrs time of sperm incubation. In regard to the water used for culture media preparation, there was no significant differences found between the two types of water, since both exhibit toxicity at 24 hrs time only. Non sterile gloves began to show toxicity at the zero time, but the sterile gloves caused toxic effect on human sperm after 1 hr time. Conversely, the two types of catheters did not exhibit toxic habit through the first 2 hours, but did after 24 hrs time of incubation. In regard to alcohol, the 0.2 mL show toxic effect at 2 hrs time, while 0.1 mL volume did not*

*show toxicity through the same period of incubation. In conclusion, HSA, BSA, sucrose, DW, MQW, as well as IUI-, and ET catheter are non sperm toxic and may be used safely in the field of ART, while the mannitol is toxic, and we preferred declining its use in ART laboratories. Further, both of the sterile and non sterile gloves are very toxic. However the sterile one is less toxicity than the non sterile. Hence, according to this study we advise using of sterile gloves in the ART laboratories if it necessary, and it must be more carefully. The results showed also that alcohol (ethanol) has deleterious effect on the human spermatozoa for short exposure time.*

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معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : زينة مزهر حسين حمد

بإشراف : الاستاذ الدكتور سعد صالح الدجيلي

التاريخ : 2009

*Effect of glycyrrhiza globra extract on in vitro sperm activation and embryonic development following intra- peritoneal insemination in mice*

## SUMMARY

*The present study aims at investigating the possibility of using Glycyrrhiza glabra extract for in vitro sperm direct activation, and its effect on in vivo fertilization rate (FR) and early embryonic development (ED) following intra-peritoneal insemination (IPI). The mice are used as an experimental model for mammals. Glycyrrhiza glabra (Gg) extract (2 mg/ml culture medium) is used for in vitro direct sperm activation technique. The female mice are divided into two groups: spontaneously ovulated (SOM) and superovulated groups (SuOM); each group is divided into two subgroups, the first : the IPI is accomplished by epididymal sperm activated in vitro by adding 20%Gg to the culture medium .The second subgroup: the IPI is accomplished without adding Gg to the culture medium. The in vitro activation of epididymal sperms with 20% Gg has shown positive effects on sperm concentration, sperm motility, and grade activity of progressive forward movement. There was a significant ( $P<0.05$ ) increased in FR with adding 20%Gg compared without adding Gg of spontaneously ovulated groups after 24 hours of insemination. The FR in the right oviduct showed highly significant ( $P<0.001$ ) increment compared to the left. Embryonic development rate significantly ( $P<0.05$ ) increased after 24 and 48 hours of insemination in grouping with adding Gg. The study showed that the quantity and quality of embryos generated from IPI with adding Gg higher than that of groups inseminated without adding Gg. It is concluded from the results of the present study that adding the 20% Gg to the*

*culture medium of the epididymal sperm and IPI leads to an improvement in the certain sperm function parameters and supports the FR in SOM and SuOM with an increase in the embryonic development rate. These data can be utilized for artificial insemination programs.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : ابتسام راضي ثامر

بإشراف : الاستاذ الدكتور أنعم رشيد الصالحي

: الاستاذ المساعد الدكتور حيدر جواد مبارك

التاريخ : 2009

*Embryonic developmental changes and dynamics of the cerebellum in chick embryo (Gallus gallus)*

## SUMMARY

*This study deals with the embryonic developmental changes that take place in the dorsal metencephalon of the chick embryo (Gallus gallus). It covers the period from 4½-5<sup>th</sup> day until day before hatching of the embryonic development. The main aims of this study are to investigate the quantitative embryonic changes at early stage in the dimensions of the cerebellar anlage. It also evaluates the major events of fissure formation in the cerebellum and the dynamics and developmental changes of the cerebellar cortex. This study was done on (60) chick eggs that were incubated in controlled environment of temperature and humidity. Embryonic development was followed up through Hematoxylin and Eosin stained paraffin sections. Staging of Hamburger and Hmilton was used. Morphometric evaluation is performed using eye piece reticle with standard calibrations. The results of this study showed that during the period from 4½-5<sup>th</sup> day to 8<sup>th</sup> day chick embryo, the transverse diameter of the cerebellar anlage increases as the embryo progresses in its development, whereas the rostro- caudal diameter decreases. This is an important stage of morphogenesis of the cerebellum, which is characterized by preliminary thickening of the anlage, which is an essential step in preparing the cerebellar anlage for development of the fissures and sulci that extend through the thickness of the developing anlage. This study reveals that the process of fissure formation takes place during the period from 9-13 days of the embryonic development. Enfolding*



*and fissure formation are proposed to be related horizontal growth of external granular layer. The process of fissure formation passes through multistage, chronologically paced steps. The morphometric evaluation shows that the external granular layer of the primitive cerebellar cortex starts to*

*regress on the day 11<sup>th</sup> of the embryonic development and ultimately disappears after hatching, after completing its role in being the source of cells of the developing cerebellum and its role in the enfolding mechanism that leads to fissure formation. The internal granular layer shows vertical growth particularly after the day 15<sup>th</sup> of the embryonic development leading to permanent histogenesis of the cerebellum.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : ابتسام جاسم سوداني

بإشراف : الاستاذة باسمه محمد عبد اللطيف الجبوري

التاريخ : 2009

*Embryonic development changes in the gonads of male mice associated with lead administration*

## SUMMARY

*The biological systems of human in the modern world are increased being exposed to lead which exists in the environment. Women at reproductive age and pregnant are more susceptible to the danger of environmental lead pollutant leading to infertility. Furthermore the developmental organs and tissues of embryos are thought to be affected by prenatal lead exposure leading to growth abnormality, spontaneous abortion and congenital problems. As there is an increase usage of electrical generators that depend on lead-based gasoline by Iraqi people which lead to increase the air pollution with this toxic substance induced us to detect through this study its possible negative effects on the fate of conception and embryonic development using the mouse as a model. This study aims to asses the effect of low dose consumption of lead acetate given to pregnant female mice on the development of gonads of male mice embryos at different periods of gestation. Mature mice aging 8-10 weeks (180 mice) weighing 25-27 grams were used. The animals were divided into three major experimental groups (G1, G2, G3) according to the level of the dose (30 animals/group), paralleled with three control groups (30 animals/group). Each major group subdivided into three minor groups (10 animals/ group) according to different periods for sacrificing during gestation period (day 14, day17, and day 20). The first experimental group (G1) administrated 0.1 mg/kg body weight (b. wt.) daily with lead acetate dissolved in 0.1 ml of normal saline intraperitoneally for 14, 17, 20 day of*

gestation while the control group was injected the same dose for the same periods with normal saline only. The second and third experimental groups (G2) and (G3) were injected with 0.2 and 0.4 mg/kg b. wt. respectively with the same route and periods for administration as in (G1), while the corresponding control animals received normal saline only for similar periods.

Vaginal smears were taken from all animals daily until metestrus phase and mating was occurred using one male mouse for each female, the first day of pregnancy registered, indicated by presence of vaginal plug. Mother's body weight, weight of uterus, weight and numbers of fetuses in right and left horn, pregnancy outcomes (abortion, and stillbirth), and diameter of fetal testes were recorded after 14, 17, and 20 day post coitum (dpc). The data revealed that administration of 0.1 mg/kg b. wt. (G1) of lead acetate caused significant decrease ( $P < 0.05$ ) in mother's weight, at 14, 17 and 20 dpc as compared with control group. At the same dose no difference was recorded at 14 dpc in uterus weight while a significant decrease ( $P < 0.05$ ) in uterus weight at day 17, and highly significant ( $P < 0.01$ ) at day 20 was recorded compared to that of control group. There was significant decrease ( $P < 0.05$ ) on weight and number of fetuses at 14, and 17 dpc at the level of this dose and highly significant ( $P < 0.01$ ) on day 20, compared to control group. With dose of 0.2 mg/kg b. wt. (G2), the results showed that there was significant reduction ( $P < 0.05$ ) in mother's weight at 14, and 17dpc, but highly significant decrease ( $P < 0.01$ ) at 20 dpc in comparison with control group. Weight of uterus at the same dose revealed significant decrease ( $P < 0.05$ ) at 14 dpc and highly significant ( $P < 0.01$ ) at 17, and 20 dpc, compared to control group. The weight and number of fetuses were significantly decreased ( $P < 0.05$ ) at 14 and 17 dpc, and reduction in these parameters became highly significant ( $P < 0.01$ ) at 20 dpc in comparison with that of control group. With a dose of 0.4 mg/kg b. wt. (G3) of lead acetate, the results showed significant decrease ( $P < 0.05$ ) in mother's weight at 14, and 17 dpc and highly significant decrease ( $P < 0.01$ ) at 20 dpc compared with control group. Weight of uterus was significantly decreased ( $P < 0.05$ ) at 14 dpc and highly significant ( $P < 0.01$ ) at 17, and 20 dpc, compared with control group. Also a significant reduction ( $P < 0.05$ ) in weights and number of fetuses in left horn at 14, 17 and 20 dpc was recorded. While in right horn, the weight and number of fetuses reveal significant decrease ( $P < 0.05$ ) at 14, and 17 dpc, highly significant ( $P < 0.01$ ) at 20 dpc

*compared with control group. Administration of 0.1 mg/kg b. wt. (G1) of lead acetate causes significant increase ( $P < 0.05$ ) in the diameter of the testes at 14, 17 and 20 dpc as compared with control group, while a dose of 0.2 mg/kg b. wt. (G2) caused significant decrease ( $P < 0.05$ ) in the diameter of the testes at 14 and 17dpc but highly significant reduction ( $P < 0.01$ ) at 20 dpc with a dose of 0.4 mg/kg b. wt. (G3) of lead acetate highly significant decrease ( $P < 0.01$ ) in the diameter of the testes was recorded at 14,17 and 20 dpc. Results showed that there is a clear association between lead and adverse pregnancy outcomes. At early period of gestation no spontaneous abortion was recorded with low dose of lead acetate (0.1 mg/kg b. wt.) but higher percentage of abortion appeared with the high dose, while the stillbirth cases mostly recorded with the dose of 0.2 mg/kg B. Wt. Even with the low dose (0.1 mg/kg b. wt.) there were a percentage of 20% cases of stillbirth recorded. As the pregnancy advanced, the number of stillbirth fetuses*

*increased with the low dose of lead acetate (0.1 mg/kg b. wt.) reaching about 50% of the total number at 20dpc. Histological study showed an important structural change in the testes of male mice embryos that belong to mothers treated with different doses of lead acetate at different periods of gestation. Obvious histopathological changes were observed in testicular sections of male embryos. These changes were increased with the increasing of dose and periods of injections at each concentration, including; disrupting the organization of testicular structure, degeneration of primordial germ cells with absence of basal lamina and pretubule myoid cells. Undescending of the testes to its normal position was observed in experimental groups (G1), (G2) and (G3) at 17 and 20 dpc. It remained adjacent to the kidneys at the upper part of the abdominal cavity while it was normally relocating at the base of the abdominal cavity in control group at 17 dpc and descent into inguinal canal at 20 dpc. It was concluded from these results that lead acetate given to pregnant female mice impaired the gonads of male mice embryos with reduction in mother's body and uterus weights along with adverse pregnancy outcomes.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : أسامه اسماعيل مجبل عباس

بإشراف : الاستاذ المساعد الدكتور محمد باقر محمد رشاد فخر الدين

التاريخ : 2009

*Comparative study on the effects of sildenafil citrate treatment on parameters of human and mice spermatozoa*

## SUMMARY

*The objectives of the present study are to investigate the effects of different concentrations of sildenafil citrate on the outcome of in vitro human sperm activation of asthenozoospermic patients, and to assess the effectiveness of orally administrated sildenafil citrate on sperm parameters of the male mice.*

*One hundred fifteen infertile patients were shared in this study. The age mean of infertile subjects was  $35.25 \pm 0.81$  years with the mean duration of infertility  $4.98 \pm 0.31$  years. Modified Earl's medium (MEM) was used only as a control group and two treated groups with different concentrations of sildenafil citrate (250 and 500 ngm/mL) for in vitro sperm activation (ISA). According to the type of ISA, seventy semen samples were prepared using direct swim-up technique and forty five semen samples were prepared using centrifugation swim-up technique. In the experimental work, one hundred twenty of healthy male mice at the age (7-8) weeks were enrolled in this study. They were divided into five major groups (24 mature male mice/group) according to different doses of sildenafil citrate (0 mg/Kg, 2mg/Kg, 4 mg/Kg, 8 mg/Kg and 16 mg/Kg) throughout administration periods for one, three and five weeks (8 males for each period). The results of the present study showed a significant improvement ( $P < 0.05$ ) in the sperm motility after in vitro human sperm activation using both concentrations (250 and 500 ngm/mL) of the sildenafil citrate when compared with the control group. Moreover, a lower concentration (250 ngm/mL) sildenafil citrate showed a significant enhancement in the progressive*

*sperm activity percentage using the direct swim-up technique better than the centrifugation swim-up technique .VIII In the male mice, a significant improvement was obtained in the sperm motility using lower doses (2 mg/Kg and 4 mg/Kg) of the sildenafil citrate compared with the other treated*

*doses and control groups. Furthermore, the results of sildenafil citrate administration showed that the shorter period had better results than the longer period on the sperm motility.*

*From the results of the present study, it was concluded that the low concentration of sildenafil citrate is effective in the enhancement of sperm motility during in vitro human sperm activation. Also, low doses and the short period of sildenafil citrate administration cause improvement in the mouse sperm motility.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : نور نوري عبد مطرود الشمري

بإشراف : الاستاذ الدكتور محيسن حسن عداي

التاريخ : 2010

*Studies on the effect of Tribulus terrestris on embryonic development in mice*

## SUMMARY

*This study was conducted to investigate the effect of aqueous extract of Tribulus terrestris (TT) on female reproductive system and embryonic development in mice. One hundred and thirty five mature males and females mice were used in this study. Thirty females and ten males were used for each dose level (100,200,300 mg/kg /day) of TT. Ten females and five males were used as control (received distilled water). The extract was given with drinking water for a period of four weeks with respect to the females and five weeks to the males. During this period the animals were weighted in weekly intervals. At the end of the 4<sup>th</sup> week of treatment (females) the animals were mated and the pregnant animals were killed at day 18<sup>th</sup> of pregnancy and embryos, uteri, ovaries were dissected out and weighted. Ovaries were kept for histological sectioning. The results showed a highly significant increase in the body weight especially in the dose levels (200 and 300mg/kg/day) compared to the control group. A remarkable increase in the reproductive organs weight (ovaries and uteri) and weight of embryos was obtained in treated groups in comparison with the control group. The histological sectioning of ovaries showed obvious increase in the diameter of corpora lutea in comparison with the control group. The result also showed significant increase in the number of embryos and corpora lutea when compared with the control group. The results of the present study demonstrated positive effect of TT on the studied fertility parameters in albino females mice.*



جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : عصام محمد جابر

بإشراف : الاستاذة باسمه محمد عبد اللطيف الجبوري

التاريخ : 2011

*Effect of superovulation induction in aged mice on fetal gonadal development and pregnancy outcome*

## SUMMARY

*The aim of this study to detect the effect of superovulation induction and in vitro fertilization in aged mice, on fetal male gonadal development and embryo quality, using the mice as a model for human .Sixty female mice divided into three groups according to their ages: G1/ 3 months,G2/10 months and G3/12 months old. All the female mice were injected intraperitonealy with 7.5 I.U. of Pregnant Mare Serum Gonadotropin (PMSG) and after 48 hours with 7.5 I.U. of human Chorionic Gonadotropin (hCG).In experiment 1: After 12-15 hours from last injection, they were sacrificed and pick up for the oocyte from the oviduct were done, three mature male mice were sacrificed to take the sperms from their epididymus which were activated by swim up techniques and used for Intracytoplasmic Sperm Injection (ICSI) , to fertilize the ova of all age groups .The fertilized oocyte incubated in CO2 incubator with Hams F-12 medium for 48 hours to let the embryos reach 4-8 cells stage .Embryos quality were examined for all groups using dissecting microscope . The result showed an inverse correlation with highly significant differences between maternal age and embryos quality.In experiment 2: After 6 hours from injection the female mice with hCG, they were mated with young male mice and the female examined for vaginal plug, the males removed from pregnant mice cages and left to progress gestation until 18 dpc, the females sacrificed and fetuses were pulled out and histological section prepared using routine histological techniques*



*for it to exam in the male gonadal development with descend testis. The result showed that there was no significant correlation between maternal age and undescended testis in males fetuses although there was slight increase in the number of undescended testis in*

*the developing male fetuses belonging to aged mother. In experiment 3: a retrospective study was done in United State of America, Texas, Huston IVF center. The study included 3455 embryos from different maternal ages, VIII separated depending on maternal age into  $\leq 29$  years, 30-34 years, 35-40 years and  $\geq 41$  years old groups, the study of the effect of maternal age on chromosomal abnormalities for eight chromosomes (X,Y,13,15,16,18,21, and 22), by using pre implantation genetic diagnosis (PGD). The result showed there is highly significant correlation adversely between advance maternal age and chromosome 22 while marginal effect with chromosome 21, and there is no significant result between maternal age and others chromosomes (X,Y,13,15,16,18). The study concluded that the advanced maternal age could affect clearly the quality of the oocytes and early embryonic stages which consequently led to failure of in vitro fertilization or implantation rate.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : سالي عدنان موسى

بإشراف : الاستاذ المساعد الدكتور محمد باقر محمد رشاد فخر الدين

التاريخ : 2011

*Effect of sildenafil citate on the reproductive parameters and pregnancy outcome in mice as a model for human being*

## SUMMARY

*The objective of the present study is to investigate the effects of oral administration, with different concentrations, of sildenafil citrate (SC) on some the of reproductive and embryonic parameters in the female mice.*

*In the experimental work, two hundred forty of healthy female mice at the age (10-12 ) weeks and weight (23-25) gram were shared in this study. They were divided into two major groups: natural and superovulated groups and then each major group subdivided into five minor groups (20 mature females mice/group) including one group as control, (40 mature females mice/group) according to different doses of sildenafil citrate ( 0.5 mg/kg, 1 mg/kg) throughout administration periods for 6 days and 14 days. Parameters were assessed involving numbers each of embryos, uterine gland, corpora lutea. Also, weight each of embryos, maternal uterus, and ovary, as well as, gestation period were reported. Furthermore, study the histological changes in ovary and uterus as, thickness of endometrium, hight of epithelial cells, each of diameter uterine gland and corpora lutea were evaluated using hematoxyline and eosin stains and measures performed by motic images plus program.*

*The results of the present study showed a significant improvement ( $P<0.05$ ) in the reproductive and embryonic parameters using both concentrations (0.5 and 1mg/kg) of the sildenafil citrate when compared with the control group as: numbers of embryos, weight of embryos, weight of maternal uterus, thickness of endometrial,*

*height of epithelial cell, number and diameter of uterine gland. Furthermore, significant increases ( $P<0.05$ ) in the each of weight of ovary, and number,*

*diameter of corpus luteum. As well as, a significant reduction ( $P<0.05$ ) in the gestation period, was recorded. Moreover, a concentration of (1mg/kg), sildenafil citrate showed a significant enhancement in the reproductive and embryonic parameters which were mentioned previously using the hormonally stimulated protocol better than the natural protocol with longer administration period.*

جامعة النهرين  
معهد أبحاث الاجنة وعلاج العقم  
رسالة الماجستير في علم الاجنة التطبيقي  
الباحثة : ايلاف حسن هادي  
بإشراف : الاستاذ الدكتور سعد صالح الدجيلي  
التاريخ : 2011

*Effect of oviductal flushing medium on fertilization rate and embryonic development  
using assisted reproductive technologies.*

## SUMMARY

*The objective of the present study was to investigate the possibility of using 50% of the oviductal flushing medium (OFM) for in vitro oocytes maturation (IVM), in vitro fertilization (IVF) and early embryonic development (ED) of mice. After the oocytes collection from the superovulated female mice, they were divided into two groups: one group the mature oocytes were divided into two subgroups, the first: cultured with 50%OFM after insemination by IVF or ICSI within 3-4 hours after collection (the treated group). The second subgroup: cultured with IVF medium (the control group). The second group the oocytes that matured in vitro by 50%OFM, they also divided into two subgroups, the first: cultured with 50%OFM after insemination by IVF or ICSI (the treated group). The second subgroup: cultured with IVF medium (the control group). The fertilization rate was recorded after 24 hours of insemination, while embryonic development rates were recorded after 24 and 48 hours of insemination.*

*The results indicated that the treatments with 50% OFM showed a positive effect on oocytes maturation in vitro. There was a significant ( $P<0.05$ ) increase in fertilization rate of the treated group with 50%OFM after 24 hours of insemination the mature oocytes by IVF (50.4%) and ICSI (30%) compared to IVF medium alone (34.4%) and (20%) respectively and there was a significant ( $P<0.05$ ) elevation in fertilization rate of the treated group after insemination the oocytes were*

*matured in vitro by IVF (39.2%) and ICSI (23%) compared to control group (30.8%) and (16.7%) respectively. Embryonic developmental rate was significantly increased after 24 and 48 hours of insemination. The study showed that the quantity and quality of embryos generated from the*

*treated group were superior to that of untreated group embryonic developments and in embryo grades. The results were attributed to the effect of various components of OFM, especially the carbohydrates, amino acids, ions and hormones. I I It is concluded from the present study that the addition of 50% OFM to the culture media of sperm and oocyte can enhance the fertilization rate, embryonic development and embryo quality in mice. This result can be used for other mammalian ARTs programs.*

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : رشا فؤاد جبر زهرون

بإشراف : الاستاذة باسمه محمد عبد اللطيف الجبوري

التاريخ : 2011

*Detection of the fetal teratogenic cases associated with environmental pollution with lead in some Baghdad's districts*

## SUMMARY

*This study is an attempt to detect the types and percentages of congenital abnormalities in a sample of fetuses and newborn infants in Baghdad and to know the predisposing factors to them, also to correlate between the blood lead level of the mothers and the percentage of congenital abnormalities in their fetuses and infants. During the period extending from the beginning of November 2009 to the end of March 2010, the pregnant women and the newborn children who were attending Al-Yarmuk hospital (south-west Baghdad) and Fatima Al-Zahraa hospital (north-east Baghdad) were examined and evaluated. Eighty mothers were included in this study. Their ages were ranged between 16 and 39 years, fifty of them were either pregnant with malformed fetus (diagnosed by U/S), or mother of child born with congenital anomaly. The remaining (30) mothers were considered as a control group including: Fifteen mothers were cases of recurrent abortion (3 or more miscarriages) were considered as positive control group and the other (15) were pregnant with normal and healthy baby, they were considered the negative control group. The data were collected through interviews with the mothers, depending on special questionnaire of general information about the mother, father and the fetus or infant. Then two blood samples were taken from the mothers for laboratory analysis, one to determine the level of lead by atomic absorption spectrophotometer and the other to detect the presence of infection with toxoplasma, cytomegalovirus (CMV) and rubella by serological tests. Results*

*revealed that the highest incidence of congenital abnormalities occurred in fetuses and infants belong to mothers with age group 21-25 years (32%) and fathers with age group 26-30 years (34%), and 32% of those fathers were working in occupations which included risk to environmental*

*pollution. The occurrence of congenital abnormalities in the 2nd child was the commonest one and represent 24% as compared to the other ranks of children in the family.*

*Out of fifty cases of congenital abnormalities, only six mothers (12%) of malformed fetuses and infants had history of congenital abnormalities in their family, 18 mothers (36%) were reported to have history of abortion, and 5 mothers (10%) showed history of stillbirth.*

*This results also demonstrated that, low social level, consanguinity, maternal occupation, parental habits (smoking and drinking), maternal chronic disease (hypertension and diabetes mellitus) and presence of antibodies of toxoplasmosis, CMV, and rubella in the serum of the mothers had no direct effect on the occurrence of congenital anomalies in their babies.*

*In addition, 82% of the families of malformed fetuses and infants lived in a distance of less than 50 meters from the source of pollution (traffic and power generators) and 18% lived in a distance of less than 100 meters away from the pollution source, also 42% of mothers who were pregnant with or gave birth to a baby with congenital abnormalities show blood lead level (BLL)  $\geq 25$   $\mu\text{g/dl}$ , 32% from those mothers their BLL was 10-24  $\mu\text{g/dl}$  and 26% the BLL of them was less than 10  $\mu\text{g/dl}$ . The commonest congenital anomalies were neurological ones representing 58% and especially neural tube defect (32%), followed by cardiovascular anomaly (18%), then urogenital anomalies (10%), fascial cleft and musculoskeletal abnormalities (6%) for each and finally 2% skin abnormalities. It was concluded from the results of the present study that the most important and effective risk factor which could lead to congenital abnormalities is the environmental pollution with heavy metal (lead) since 42% of babies with congenital abnormalities, their mothers showed BLL  $\geq 25$   $\mu\text{g/dl}$ , finally the neurological anomalies, especially neural tube defects, are the most common types of congenital abnormalities in*

*fetuses and newborn infants with relatively same incidence in both male and female.*



جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : علي ابراهيم محمد

بإشراف : الاستاذ المساعد الدكتور محمد باقر محمد رشاد فخر الدين

التاريخ : 2011

*Assessment of sperm chromatin structure in relation to human sperm parameters, in assisted reproductive techniques*

## SUMMARY

*Human sperm chromatin (DNA and nuclear proteins) is vulnerable to be affected by a variety of internal and external factors with various mechanisms through the journey of production and transmission of spermatozoa. The integrity of the paternal genome is therefore of paramount importance in the initiation and maintenance of a viable pregnancy both in a natural and assisted conception. The need to diagnose sperm at a nuclear level is an area that needs further understanding to improve treatment of the infertile couple. In view of that, the objectives of the present study were: to study the correlation of sperm chromatin integrity with the main seminal fluid analysis parameters (concentration, percentage of normal morphology and percentage of progressive motility) in infertile patients; to study the comparison between sperm chromatin integrity in infertile patients and that of fertile controls; to study the effect of sperm chromatin integrity of infertile patients on the outcome of intrauterine insemination (IUI); and to study the effect of sperm chromatin damage of infertile patients, involved in intracytoplasmic sperm injection (ICSI) program, on their fertilization and pregnancy rates.*

*This study was performed as two parts:*

### *I. Part One:*

*This study involved 75 non-azoospermic infertile patients, with primary infertility, who were involved in IUI program with mean age  $29.05 \pm 0.58$  years (range 20 - 40*

years) and mean duration of infertility period  $3.75 \pm 0.28$  years (range 1 – 12 years); and 20 controls (healthy fertile donors) with mean age  $29.65 \pm 0.66$  years (range 24 – 35 years). The acridine orange (AO)

test was used in this study to measure sperm chromatin integrity. The main results of this study showed that there was a highly significant correlation of sperm chromatin integrity with sperm

concentration ( $r = 0.335$ ,  $P = 0.003$ ), and with percentage of normal sperm morphology ( $r = 0.482$ ,  $P < 0.001$ ). There was non significant correlation between sperm chromatin integrity and percentage of progressive sperm motility ( $r = 0.037$ ,  $P = 0.756$ ). In addition, there was a highly significant inverse correlation of sperm chromatin integrity with duration of infertility ( $r = -0.404$ ,  $P < 0.001$ ) and with age ( $r = -0.296$ ,  $P = 0.010$ ). Also, the infertile patients had a highly significant poorer sperm chromatin integrity as compared with the controls ( $35.867 \pm 1.540$  vs.  $60.850 \pm 2.174$ ,  $P < 0.001$ ). The IUI negative pregnancy patients, as compared with patients with IUI positive pregnancy, had highly significant poorer sperm chromatin integrity ( $31.688 \pm 1.126$  vs.  $60.182 \pm 1.981$ ,  $P < 0.001$ ).

## **II. Part Two (research fellowship/Germany):**

This study involved 50 non-azoospermic infertile patients who included in ICSI program with mean age  $38.22 \pm 0.72$  years (range 26 - 55 years). The TUNEL assay was used in this study to measure sperm chromatin damage.

The main results of present study showed that, regarding sperm chromatin damage of patients with ICSI negative pregnancy in comparison with patients with ICSI positive pregnancy, there was non significant difference between them ( $24.51 \pm 1.09$  vs.  $24.06 \pm 1.05$ ,  $P = 0.810$ ). Also, there was non significant correlation between sperm chromatin damage and the fertilization percentage post-ICSI ( $r = -0.013$ ,  $P = 0.928$ ).

جامعة النهرين

معهد أبحاث الاجنة وعلاج العقم

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : بان ثابت سعيد

بإشراف : الاستاذة باسمه محمد عبد اللطيف الجبوري

الأستاذ المساعد الدكتور سعد محمد ندا

التاريخ : 2011

*Histological and cytogenetic effects of oral contraceptive pills during pregnancy  
on some aspects of fetus and newborn in mice*

## Summery

*Two experiments were conducted to investigate the effect of normal (0.034 mg/kg body weight, B. Wt) and double dose (0.068 mg/kg b. wt.) of oral contraceptive pills (OCPs) that contain steroid hormones as a low dose of ethinyl- estradiol (0.03mg) and high dose of levonorgestrel (0.15mg) that given to pregnant female mice on the development of their male fetuses gonads as well as on some cytogenetic attributes (mitotic index and chromosomal aberrations). In experiment 1, ninety mice of 8-10 weeks age and 25-30gm weight were randomly divided into 3 equal groups (30 mice each). The first (G1) and second (G2) groups were orally administered with 0.034 and 0.068 mg/kg b. wt. per day of OCPs respectively, while the third group regard as control administered with distilled water. Each major group were subdivided into minor groups (15 mice/subgroup) according the period of sacrificing during gestation (day 17 and birth). The G1 and G2 animals exhibited the lower ( $P < 0.01$ ) weight and number of fetuses during day 17 and at birth periods, as compared with control group. High percentage of spontaneous stillbirth was noted in G2 group (15.58%), followed by G1 animals (5.50%) as compared with control group (2.44%). On the other hand, high dose of OCPs (G2) was significantly ( $P < 0.01$ ) decreased the testes diameter of fetuses at day 17 and at birth, whereas, the G1-*

*group exhibited low ( $P<0.01$ ) testes diameter of fetuses at birth only in comparison with control group. In experiment 2, thirty*

*mature female mice of 8-10 weeks age and 25-30 gm B.Wt. were randomly divided into 3 groups (10 mice/group). The G1 and G2 groups were administered orally with 0.034 and 0.068 mg/kg b. wt. per day of OCPs respectively for 14 days during gestation, while the control group administered distilled water only for the same period. Two weeks post-birth, 10 males/group were taken to study the chromosomal aberration on bone marrow and spleen cells and mitotic index. Results indicated that OCPs lead to reduction in mitotic index and induced spontaneous chromosomal aberration in treated mice. In conclusion, the steroid hormones given to pregnant female mice impaired the gonads of their male fetuses as well as lead to some cytogenetic impairment.*

جامعة النهرين

المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : خالد عبد الكاظم ثامر

بإشراف : الاستاذ الدكتور انعم رشيد الصالحي

الأستاذ الدكتورة استبرق عبد الرسول الواسطي

التاريخ : 2011

## Effect of Vasectomy on testicular tissue and DNA Changes in Mice

### Summary

*Vasectomy is a simple, effective, and widely used method of birth control of male for many years. With the development of microsurgery, this previously permanent procedure now can be reverse and due to an increasing demand for reversal of vasectomy, there is a growing interest in research on the effects of vasectomy on the testis and in whether or not these changes may be reversible.*

*Within such context, the present study was designed to investigate the alteration in the histology of the testis after vasectomy as well as determine the effect of the operation in provoked DNA damage in testicular tissue in the male mice as a human model.*

*Thirty mature male mice where used in our study. The mean age of mice was ten weeks. Ten of them are consider as control group and other twenty as treated group. On treated group, we perform bilateral vasectomy, and after six weeks, sacrificed the mice and took the testes to evaluate the change that occurred after vasectomy. Histological evaluation was done by paraffin section and semi thin section, and biochemical evaluation was done by single cell gel electrophoresis (comet assay).*

*The microscopical observation of slides obtained from testis for vasectomized group show obvious alterations summarized as; degeneration of spermatids, thickened of basement membrane, dilatation of the seminiferous tubules, reduction in the seminiferous cell population, oedema of the intertubular spaces and interstitial fibrosis.*

*Biochemical results of the present study showed a highly significant ( $P<0.0001$ ) increase in DNA damage among vasectomized mice (46.02%) compared with control group (27.17 %) after six weeks of operation.*

*From the results of the present study, it was concluded that there was alteration in testis histology after vasectomy, low level of DNA damage (as expressed by comet assay) was reported among healthy control mice and vasectomy was associated with DNA damage in testis tissue.*

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المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : رنا عبد الرسول

بإشراف : الاستاذ الدكتور انعم رشيد الصالحي

التاريخ : 2011

**A Correlative Study of  
Biochemical Profile of Contractile and Collagenous Proteins and Histogenesis during  
Embryological Development of Selected Skeletal Muscles**

## **Summery**

*This is a correlative study of embryological and biochemical changes that takes place during the development of the back skeletal muscles of the rat as a mammalian model. Experimental embryological study was done on the histogenesis of back skeletal muscles starting from embryonic stage 17 till new born stage. The morphological and histological study was accompanied by a biochemical study of the levels of contractile proteins in the tissue using Lowery assay and the level of collagenous proteins evaluated by Reddy and Enwemeka assay.*

*It was demonstrated that myogenesis is a dynamic process that continues till birth. The process of organization of muscle runs in two simultaneous pathways of organization of connective tissue into epimysium, perimysium and endomysium and the organization of muscle fiber by growth of their cytoplasm and rearrangement of their nuclei.*

*Myogenesis passes through consecutive, sequential steps that lead to fusion of myotubes and the formation of muscle fibers, formation of satellite cells and reinitiating of the process of myogenesis.*

*The increase in the tissue level of contractile proteins in the developing muscle is accompanied by a decrease in the tissue level of collagenous proteins that starts after the embryonic stage 17 of development.*

*The relative proportion between contractile proteins in developing muscle tissue and that of collagenous proteins at embryonic stage 17 was (1:2) becomes inverted (2:1) when estimated in new born rat.*

*The muscles of the back become segregated into well defined morphological groups of epixial and hypaxial muscle in the embryo before birth.*



جامعة النهدين

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رسالة الماجستير في علم الاجنة التطبيقي

الباحث : عبد الفتاح كاظم طاهر

بأشراف : الاستاذ الدكتور سعد صالح الدجيلي

التاريخ : 2011

**Effect of Pentoxifylline, L-Carnitine and ovulation induction  
on oocyte quality and embryonic development using assisted**

**Summary**

*The objective of the present study is to find out the possibility of decreasing the defects of superovulation (SUO) medicine in the oocyte quality and embryonic development(ED). The study has examined the -effect of the following drugs were tested to overcome these defects:1*

*Effect of pentoxifylline (PTX) and L-carnitine (LC) with SUO medicine (on ovulation induction program. 2- Effect of the pentoxifylline (PTX and L-carnitine (LC) with SUO medicine on the oocyte quality and ED .using assisted reproductive technology (ART) for insemination*

*Hams-F12 was the culture medium used for in vitro direct sperm activation technique, in vitro fertilization (IVF) and ED following 24-48 hours of insemination. According to the SUO program and type of treatment, the female mice were randomly divided into 5 groups (10 mice for each group) and treated intr-peritoneally (IP) as follow: Group*

*(injected with normal saline for 10 days, considered as control (C -:1 group, 64 oocytes were collected from it, group 2:-injected with normal saline for 10 days and given SUO medicine, 160 oocytes were collected from it, group 3:- injected with PTX for 10 days and given SUO medicine, 206 oocytes were collected from it, group 4:- injected with LC for 10 days and given SUO medicine, 144 oocytes were collected from it group 5:- injected with PTX and LC for 10 days and given SUO medicine, 305 oocytes were collected from it. The oocytes of each group (were inseminated by IVF with activated sperm. The fertilization rate (FR was recorded after 24 hours of insemination, while ED rates were .recorded after 24 and 48 hours of insemination*

*The results indicated a highly significant ( $P<0.001$ ) difference in the number of collected oocytes between control and treated groups. There was a highly significant ( $P<0.0002$ ) increase of G3 and a highly significant ( $P<0.0008$ ) increase of G5 was reported in the FR and compared to G1. A highly significant ( $P<0.0001$ ) increase was reported in the FR of G3 and G5 compared to G2. The ED rate of 3-4 and 5-8 cells was a highly significantly ( $P=0.007$ ) in G5 compared to G 2 after 48 hours of insemination. The study showed that the quantity and quality of embryos generated from the treated groups G3 and G5 were superior and .significant of other groups in ED and in embryonic grades*

*It is concluded from the present study that the treatment with PTX*

*and LC through SUO program enhance the FR, ED and embryonic quality in mice. This result can be used for other mammalian ARTs programs.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحث : حكيم جواد كاظم

بإشراف : الأستاذ الدكتور

انعم رشيد الصالحي

الأستاذ المساعد الدكتور

استبرق عبد الرسول الواسطي

التاريخ : 2012

## **Evaluation of DNA Fragmentation in the Testicular Tissue after Cryopreservation/Thawing cycle using Comet Assay**

### **Summary**

*Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, such as (typically)  $-80^{\circ}\text{C}$  or  $-196^{\circ}\text{C}$  and this process use extensively in programs of in vitro fertilization (IVF).*

*Now with recent improvement of assisted reproductive technologies and possibility of using testicular spermatozoa or epididymal spermatozoa at in vitro fertilization by intracytoplasmic sperm injection (ICSI), the cryopreservation of testicular tissue become widely used in male who were treated by chemotherapy or radiotherapy because these therapeutic applications can lead to sterility.*

*This study is an attempt to evaluate DNA fragmentation in the cell after cryopreservation/thawing cycle when using different types of cryoprotectant (CPA) as well as to investigate the changes that occur in mice testicular tissue after cryopreservation/thawing cycle as a model for humanbeing.*

*Sixty mature fertile male mice were used in the current study, the mean age of these mice were ten weeks. Fifteen of them were considered as control group and the rest*

*(forty five) as cryopreserved group, this group was divided into three subgroup according to the type of cryoprotectant (glycerol, 1, 2 propanediol and dimethylsulfoxide), each subgroup composed from fifteen mice. The testes were cryopreserved for six weeks then histological evaluation was done by paraffin section and DNA fragmentation assay was done by single cell gel electrophoresis (comet assay).*

*The microscopically observation of slides obtained from cryopreserved testis differs according to the type of cryoprotectant, in the dimethylsulfoxide group the tissue appeared well-preserved cell and mild changes in the interstitial tissue, but the glycerol group shown moderate changes in testicular tissue after cryopreservation such as; rupture of the stroma, detachment the cells form basement membrane in the seminiferous tubules, gaps in germinal epithelium and folding in the lamina propria, while in the 1,2 propanediol group the tissue shows sever changes such as distraction the interstitial tissue , necrotic cells in the seminiferous tubule and difficult to recognize the cells in the seminiferous tubule.*

*Comet results of the present study showed a highly significant ( $P < 0.0001$ ) increase in DNA damage in the cryopreserved testis (33.26%, 38.8% and 30.6% represent cryoprotectant glycerol, 1, 2 propanediol and dimethylsulfoxide respectively) compared with control group (23.06%) after six weeks of cryopreservation.*

*From the results of the current study, it was concluded that there was alteration in testis histology after cryopreservation/thawing cycle and increase the level of DNA fragmentation after cryopreservation/thawing cycle. As well as dimethylsulfoxide cryoprotectant provide good protection for testis histology and DNA.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحث : طيف كريم خلف

بأشراف الأستاذ الدكتور

انعم رشيد الصالحي

التاريخ : 2012

## The Effects Of Glucocorticoids On Embryonic Development Of The First molar Teeth Of Rat

### Summary

#### Background:

*Corticosteroids are important steroids, well known for their anti-inflammatory and immunosuppressive properties. They are widely used in chronic inflammatory disease and in a wide spectrum of immunological disorders. Hydrocortisone and prednisolone are commonly used members of these steroids.*

*This study is concerned with effects of glucocorticoids on the embryonic development of teeth in rats.*

*The first stage of the formation of teeth is the formation of dental lamina which subsequently bud into the underlying mesenchyme. During teeth development these stages are recognized which are the bud, cap and bell stages. Then cusp formation starts and distinct shape of the tooth is established. Formation of the tooth structure is accomplished by odontoblasts and ameloblasts secreting dentin and enamel.*

### *Aim of the study*

*The main objective of this study is to evaluate the effects of the prenatal administration of specific glucocorticoids (Hydrocortisone and Prednisolone) on the teeth development and its impact on the morphology of the teeth developmental stages and explore the teratogenic effect of these steroids.*

### *Materials and Methods:*

*This study was carried out on rat embryos as a model of mammalian embryo with teething pattern of molar teeth similar to that of the human. Three group, each included (64) pregnant rat, one was a control group, the second group received 50 body weight daily/kg/mg daily dose hydrocortisone I.M., third group given 0.5 mg*

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الباحث : نبأ عفيف عبد العزيز  
بأشراف الأستاذ الدكتور  
سعد صالح الدجيلي  
التاريخ : 2012

The effect of *Citrullus colocynthis* medium on fertilization rate and early cleavage stages of mice embryos using assisted reproductive technology

### *Summary*

#### *Back ground*

*Citrullus colocynthis* is an herbal medicine used in the treatment of a wide range of diseases. Recently, the effect of this plant on the reproductive system has been studied .However, its role in in vitro still unclear.

#### *Objectives*

The present study aim to investigate the possibility of using *Citrullus colocynthis* extract for in vitro sperm direct activation and its effect on in vitro Fertilization rate (FR) and early embryonic development(ED) following in vitro insemination process.

#### *Materials and Methods*

*Citrullus colocynthis* extract (0.5mg/ml culture medium) was used for in vitro direct sperm activation technique. The female mice were divided into two groups, the first group



*inseminated with epididymal sperm activated in vitro by adding 10%CC to the culture medium and incubated with 10%CC-Ham, s F-12 medium (the treated group) while, the second group inseminated with epididymal sperm activated in vitro without adding 10%CC to the culture medium and incubated with CC-free Ham's F-12 medium.*

### **Results**

*In vitro activation of epididymal sperms with 10%CC has shown positive effect on sperm concentration, sperm motility, and grade activity of progressive forward movement. There was a significant ( $P<0.05$ ) increase in FR with adding 10%CC compared with that free of CC of control group after 24 hours of insemination in grouping with adding CC. The study showed that the quality and quantity of embryos generated from treated medium (with 10%CC) were higher than that of group inseminated without adding CC.*

*Conclusion: It is concluded from the results of the present study that adding the 10%CC to the culture medium of the epididymal sperm and in vitro inseminated lead to an improvements in certain sperm function parameters and supports the FR and early embryonic development rate.*

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رسالة الماجستير في علم الاجنة التطبيقي  
الباحث : رعد حسين أحمد الموسوي  
بإشراف الأستاذ الدكتور  
محمد باقر محمد رشاد فخرالدين

التاريخ : 2012

**Vitrification Effect on Morphology and Viability of Oocytes Using Different Cryoprotectant Agents**

*Summary*

***Background:***

*Oocyte vitrification is a promising technique. Immature oocytes vitrification has been emerged to avoid the risk of meiotic spindle damage that may be encountered during mature oocyte vitrification. The choice of appropriate types and concentrations of cryoprotectants is essential for the success of the oocytes vitrification.*

***Objectives:***

*This study aimed to investigate the effects of vitrification on viability and morphology of*

*oocytes and to compare the effect of several cryoprotectants on the viability and morphology of oocytes during vitrification and post-thawing.*

***Materials and Methods:***

*395 oocytes were normal and viable oocytes included in this study. By using cryotop, immature oocytes that were viable with normal morphology were vitrified with 15% DMSO (Dimethyl Sulphoxide) and either 15% EG (Ethylene Glycol). or 15% PrOH (1, 2-Propanediol) supplemented with 0.0 M as control group, 0.25M, or 0.5 M of either sucrose or trehalose for treated groups. Oocytes viability and morphology were assessed post-aspiration and post-thawing.*

***Results:***

*Results of the present study showed that despite the percentage of post-thawing normal and viable oocytes observed with the use of 0.5 M of either sucrose or trehalose were higher than that found with 0.25 M of either sucrose or trehalose for all other treated groups, the differences were not significant ( $P > 0.05$ ). Non significant differences ( $P > 0.05$ ) in the percentage of post-thawing normal and viable oocytes were noticed between the use of either EG or PrOH.*

***Conclusion:***

*Vitrification is simple technique and easy to perform, but it needs some experience to prevent any oocyte loss during vitrification and thawing. The use of 0.5 M of either sucrose or trehalose in vitrification solution improves the percentage of post-thawing viable and normal oocytes.*

جامعة النهرين

المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : زينب فائق رزيق

بإشراف الأستاذ المساعد الدكتور

نوال خيرى العاني

التاريخ : 2012

Evaluation of certain tumor markers (PSA,CA125 and CA15-3)in some Iraqi women with Polycystic Ovarian Syndrome.

### Summary

#### **Background:**

*Polycystic ovary syndrome (PCOS) is the most common cause of hyperandrogenism anovulatory infertility; it affects 5-10 % of females in the reproductive age. PCOS is a risk disease , it has an association with gynecological malignancy.*

#### **Objectives:**

*To determine whether serum Prostate Specific Antigen(PSA),Cancer Antigen 125 (CA125) and Cancer Antigen 15-3(CA15-3) levels are increased in PCOS and possibility of PSA,CA125 and CA15-3 to be used as diagnostic marker of PCOS.*

#### **Method:**

*Seventy females in their reproductive age (20years-40 years) with PCOS diagnosed depending on three criteria :*

*Menstrual history of oligomenorrhea , Ultrasound revealed polycystic ovaries and Clinical or Biochemical hyperandrogenism.*

*Twenty normal fertile females who served as control group in this study.*

*Blood samples were aspirated from all individuals from 2nd-4th day of menstrual cycle to measure FSH, LH and testosterone levels ( by Mini VIDAS assay). Other blood samples were collected from the same patients and controls during late follicular phase to measure total PSA ,CA15-3 and CA125( by enzyme linked immunosorbent assay ) .*

***Results:***

*Patients with PCOS had significant increase in PSA and Total serum testosterone ( $p < 0.05$ ).*

*Patients with PCOS had highly significant increase ( $p < 0.001$ ) in LH level and highly significant increase ( $p < 0.001$ ) in LH/FSH ratio and BMI parameters.*

جامعة النهرين

المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

الباحث : مهند ساجت عويد الدلفي

بإشراف الأستاذ الدكتور

سعد صالح الدجيلي

رسالة الماجستير في علم الاجنة التطبيقي

الاستاذ الدكتور عبد الحسين مويت الفصيل

التاريخ : 2012

**Effect of Pentoxifylline and *Glycyrrhiza glabra* activation on cryopreserved epididymal sperms**

*Summary*

*Background: Many studies conducted on the pentoxifylline (PX) and Glycyrrhiza glabra (G.g) as motility stimulants showed positive effect on the activation of sperm in vitro and improve the forward movement. However, it was not known the impact of these stimulants on the nature of genetic material, especially after cryopreservation.*

*Objective: This experiment was designed to found out any harmful effect of medium contains a mixture of pentoxifylline and aqueous extract of Glycyrrhiza glabra on the sperm genetic material DNA before and after cryopreservation.*

*Materials and Methods: In this study, fifty mature fertile male mice (8-12) weeks old were used. Epididymal sperms were obtained from caudal region and direct in vitro activation technique by using four media namely; PX alone, Glycyrrhiza glabra alone, a mixture of PX with Glycyrrhiza glabra and Ham's F-12 (as control media) were performed to evaluate the sperm function characteristics as well as the evaluation of DNA normality by acridine orange and comet assay test before and after cryopreservation.*

*Results: This study showed a highly significant ( $P < 0.001$ ) improvement in certain sperm function parameters i.e the sperm forward movement ,morphologically abnormal sperms (MAS) with a highly significant ( $P < 0.001$ ) decrease in DNA abnormality using acridine orange stain and comet assay, following in vitro activation by the four media compared to the before activation. Following in vitro activation and cryopreservation, the results of epididymal sperm showed a highly ( $P < 0.001$ ) decrease in DNA abnormality by using PX medium alone, Glycyrrhiza glabra medium alone and the mixture of PX and Glycyrrhiza glabra compared to control medium and the results before cryopreservation, with a significant ( $P < 0.05$ ) decline in progressive motility by using any of four media compared to before cryopreservation.*

*significant*

*Conclusion: According to the results of present study, it has been found no harmful effect of PX and /or Glycyrrhiza glabra on the epididymal sperms DNA after activation in vitro and cryopreservation by using acridine orange stain and comet assay. The best result noticed when using a medium contains a mixture of PX+Glycyrrhiza glabra together.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحث : زيدون حسن جبار

بإشراف الأستاذ الدكتور

محمد باقر محمد رشاد فخرالدين

التاريخ : 2012

**Correlation of Antisperm Antibodies with DNA Structure and Sperm Parameter**

*Summary*

*Background: The immune infertility caused by anti-sperm antibodies (ASAs) represented about 10-20% of infertility among the couples, the ASAs) interfere with sperm parameters such as the sperm motility and sperm ability to penetrate cervical mucus, sperm-oocyte binding, and fertilization and embryo developments. In addition, the DNA damages increasingly found with infertile subjects that affect male reproduction potency and progeny.*

*Objective: The present study was designed to assess semen analysis, presence of (ASAs) and DNA fragmentation index as well as correlation within these parameters in normzoospermic Iraqi subjects.*

*Materials and Methods: A total number of Iraqi subjects (116) with range of age (20*



*51) years and their mean duration of infertility ( $4.70 \pm 2.77$ ) classified into seventy-seven with primary type of infertility and thirty-nine with secondary type of infertility.*

*The mixed agglutination reaction (MAR) test used to assess their (ASAs) in semen (direct method), seminal plasma and blood serum (indirect method); for the both IgG and IgA class of antibodies and their distribution among different parts of spermatozoa as percentage. Acridine orange test (AOT), where used to asses DNA*

*Results: The mean of IgG by direct method was ( $23.88 \pm 1.75$ ) and for indirect detection method the mean ( $27.41 \pm 2.41$ ) were no significant difference ( $P > 0.05$ ) between the two methods. While the direct method for IgA detection mean ( $14.46 \pm 1.76$ ) and the mean for indirect method of detection was ( $6.86 \pm 0.39$ ) and there was significant difference between the two methods ( $P < 0.05$ ). In addition to that, the distribution of IgG and IgA detected by direct method on sperm parts showed no significant difference except for the sperm tail; while the indirect method for IgA and IgG showed significant difference in distribution on sperm mid-piece.*

*The DNA fragmentation index for all subjects mean was ( $38.25 \pm 2.08$ ), for subjects with primary infertility type ( $41.61 \pm 2.19$ ) and subjects with secondary infertility type ( $31.63 \pm 4.29$ ).*

*fragmentation index (DFI).*

*Conclusion: The percentage of ASAs showed no difference with DFI, but the percentage of ASAs according to their attachment with sperm parts (head, mid-piece and tail) had less correlation with DFI.*

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المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

رسالة الماجستير في علم الاجنة التطبيقي

الباحث : مصطفى جواد جليل

بإشراف الأستاذ الدكتور

انعم رشيد الصالحي

و الأستاذ المساعد الدكتورة

استبرق عبد الرسول الواسطي

التاريخ : 2012

### Evaluation of Different Cryopreservation Protocols

of the Testis Using

8-Hydroxy2'-Deoxyguanosine as Marker of DNA Damage.

#### Summary

*Background: Chemotherapy and radiotherapy can destroy or severely reduce spermatogenesis and thereby jeopardize fertility in the long term. There is still no medical treatment that guarantees fertility preservation after chemo- and radiotherapy. Now with recent improvement of assisted reproductive technologies (ART) and possibility of using testicular spermatozoa or epididymal spermatozoa at in vitro fertilization (IVF) by intracytoplasmic sperm injection (ICSI), cryopreservation of testicular tissue is an option in fertility preservation for males who will lose spermatogenic cells as a result of chemo- and radiotherapy and for males with azoospermia.*

*Objectivs of the study: This study is an attempt to evaluate oxidative DNA damage in the mice testicular tissue after cryopreservation/thawing cycle when using different types of cryoprotectants as well as to investigate the changes*

*that occur in mice testicular tissue after cryopreservation/thawing cycle as a model for human being.*

*Materials and methods:* Fifty mature fertile male mice between 8-12 weeks old were used in the current study. For each mouse, one testis was evaluated histologically and immunohistochemically (using 8-Hydroxy 2'-Deoxyguanosine as marker of oxidative DNA damage) without cryopreservation (control group). The other testis was evaluated histologically and immunohistochemically (using 8-Hydroxy 2'-Deoxyguanosine as marker of oxidative DNA damage) after six weeks of cryopreservation using different types of cryoprotectants (cryopreserved group).

*Results:* The microscopically observation of slides obtained from cryopreserved testis differs according to the type of cryoprotectant (glycerol, 1,2 propanediol and dimethylsulfoxide), in the dimethylsulfoxide group, tissue sections showed no major differences versus control group, but in the histological sections obtained from tissue cryopreserved by glycerol as cryoprotectant showed moderate morphological and structural changes as compared with the control group. While, the tissue samples subjected to 1,2 propanediol as cryoprotectant displayed the severest morphological and structural changes as compared with the control group.

*Immunohistochemical results of the present study showed a highly significant ( $P < 0.001$ ) increase in oxidative DNA damage in the cryopreserved testis (46.78%, 63.45% and 32.59% represent cryoprotectant glycerol, 1, 2 propanediol and dimethylsulfoxide, respectively) compared with control group (19.27%) after six weeks*

*of cryopreservation.*

*Conclusion:* From the results of the current study, it was concluded that there was alteration in testis histology after cryopreservation/thawing cycle and increase the level of oxidative DNA damage after cryopreservation/thawing cycle. As well as dimethylsulfoxide cryoprotectant provide good protection for testis histology and DNA.

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : ضحى اكرم ابراهيم

بإشراف الأستاذ الدكتور

عدنان صالح الجنابي

الأستاذ الدكتور

محمد باقر محمد رشاد فخر الدين

التاريخ : 2012

**Effect of Penicillin on the Ovary and Endometrium in the Mice**

*Summary*

*Background: The reproductive failure is a significant public health concern. Although relatively little is known about factors affecting fertility, there is sufficient evidence to hypothesize that antibiotic may influence the fertility.*

*Objective: This study was established to explore the individual impact of different doses of penicillin on some reproductive parameters.*

***Materials and Methods:*** Forty adult female albino mice (12-18weeks) and weight (25-28) gm divided into four groups. Control group (G1) no. of mice (10) was treated daily with sterile water, and other three groups that treated with different doses of penicillin G2 (2mg/kg. B.wt/day) no. of mice 10, G3 (3mg/kg. B.wt/day) no. of mice 10, G4 (4mg/kg. B.wt/day) no. of mice 10, for four cycles twice daily (IM) of penicillin injection at proestrus phase and sacrificed at estrus phase. Parameter analysis involving body and reproductive organ weights, diameter of ovary, thickness of endometrium, length of epithelial cells, number and diameter of uterine gland, corpora leutea and follicles (preantral and antral) using histological section by (motic image plus) and hormonal assay including LH, FSH and estradiol E2.

***Results:*** The results of this study demonstrate that there is significant decrease ( $P < 0.05$ ) in organ weight of reproductive system, number and diameter of total ovarian follicles (preantral, antral), and corpora leutea, diameter of ovary, thickness of endometrium, height of epithelial cell, number and diameter of uterine glands and serum level of FSH, LH and E2 of female mature mice after treatment with high doses (4mg/kg. B.wt/day), while, there is no significant decrease ( $P > 0.05$ ) after treatment with (2mg/kg. B.wt/day); (3mg/kg. B.wt/day) comparing to control group.

***Conclusion:*** The result showed higher doses for long period of penicillin has impact of some reproductive parameters of mature female

جامعة النهرين

المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب

رسالة الماجستير في علم الاجنة التطبيقي

الباحثة : سوسن صاحب حمزة

بإشراف : الاستاذ الدكتور سعد صالح الدجيلي

التاريخ : 2102

In vitro Embryonic Development Following  
the Insemination with Epididymal Sperms of  
Vasectomized Mice Activated by the extract  
of Glycyrrhiza glabra Root

**Summary**

*Background: Many studies conducted on the Glycyrrhiza glabra (G.glabra) as motility stimulants showed positive effect on the activation of sperm in vitro and improve the forward movement. However, it was not known the impact of this stimulant on the nature of sperms parameters in vassal obstruction of males and its effect on each embryonic development when used in in vitro fertilization (IVF) program)*

*Objective: This study was designed to examine the possibility of fertilization ova by activated epididymal sperm of vasectomized mice by G . glabra as a model for obstructive azoospermia and measuring the fertilization rate (FR) and embryonic development (ED) at early cleavage stages*

*(Materials and Methods: In this study, G. glabra (0.3 mg/ml culture medium was used for in vitro direct sperm activation technique. The female mice were divided into two groups , the first group insemination with epididymal sperm ( activated by adding G. glabra to culture medium and incubated (treated group .while the second group were inseminated by epididymal sperm activated by G .glabra - Free medium . Both FR and ED were accounted*

*Results: This study showed a highly significant ( $P < 0.001$ ) improvement in certain sperm function parameters following the addition with G. glabra i.e. the sperm forward movement, and morphologically normal sperms (MNS). There was significant ( $P < 0.05$ ) increase in FR of superovulated (SUO) mice oocyte by using 30 % G. glabra medium compared to G. glabra- Free (%75.96) medium (Hams –F12 medium ) alone in SUO group (54.4%) . By adding 30% of G.glabra to the medium , the rate of 2-cell following 24hours of insemination was 60% and the rate of 3- 4-cell following 48hours of insemination 65.78% . There result were significantly ( $p < 0.05$ ) higher than that corresponently rate of ED by using G.glabra- Free medium (50.73% 2-cell and .( cell , respectively 4-3 %58.82*

*Conclusion: According to the results of present study, the investigation showed that the G. glabra may contain many factors and energy sources that supporting the growth and normal development of early cleavage stages of mice . embryos in vitro following IVF with epididymal sperm of vasectomized mice*

*This result can be utilized for IVF program in mammals.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة:- شعوب ياسين حسين

بإشراف الاستاذ الدكتور انعم رشيد الصالحي

الاستاذ الدكتور محمد باقر محمد رشاد فخر الدين

التاريخ: 2013

## Effect of Sperm Cryopreservation Protocols in Relation to Human Sperm Mitochondrial Apoptosis Activity and Sperm Chromatin Structure

### Summary

#### Background:

*Human sperm cryopreservation is a valuable technique in the field of fertility, especially, those patients who are prone to become infertile due to surgical or medical treatments such as chemo- or radiotherapy for cancer treatment. In fact, semen cryostorage seems to be the only proven method that may offer these couples a chance of having children in the future: cancer therapy could in fact lead to damage, resulting in subfertility or sterility due to gonad removal or permanent damage to germ cells caused by adjuvant therapy.*

#### Objectives:

*This study was aimed to investigate the effects of cryopreservation protocols on the human sperm parameters and the effects of cryopreservation on sperm DNA structure and sperm mitochondrial apoptosis.*

#### Materials and Methods

*Sixty semen samples were included in this study. Sperm parameters, DNA fragmentation index (DFI) and mitochondrial apoptosis (MA) were assessed pre- or post-cryopreservation. Cryopreservation was done using either 15% DMSO or 15%*



*glycerol either alone (as control group) or supplemented with either 0.25M or 0.5M of sucrose for treated groups. Crude data were statistically analyzed.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة:- ضمياء عبد نجم

بإشراف الاستاذ الدكتور محمد باقر محمد رشاد فخر الدين

الاستاذ المساعد الدكتور محمد عودة سلمان

التاريخ: 2013

Effect of several antibiotics administered to infertile patients with leukospermia on frequency of spermatogenic round cells and sperm DNA structure

### Summary

#### **Background:**

*Antibiotics are widely used in the treatment of male infertility, as well as in the different fields of ARTs. However, these antibiotics are related to some harmful effects on spermatogenic and non-spermatogenic cells.*

#### **Aims of the study:**

*The objective of this study is to find out the effects of antibiotics (Ciprofloxacin, Metronidazole and Levofloxacin) used in the treatment of male infertility on sperm parameters, sperm DNA structure and frequency of spermatogenic and non spermatogenic round cells in the seminal fluid for leukospermic men.*

#### **Materials and methods:**

*This study includes sixty two men with age 20-39 years old referred to High Institute of Infertility Diagnosis and Assisted Reproductive Technology/ Al - Nahrain University. Semen samples were collected from infertile men by masturbation and examined under light microscope for selection leukospermic semen sample. Seminal fluid analysis, peroxidase (Endtz) test and DNA fragmentation assay were done pre-treatment and post-treatment. In the presented*

*study there are three antibiotics are used (ciprofloxacin, metronidazole and levofloxacin).*

#### ***Results:***

*There is non-significant increment ( $P>0.05$ ) in semen volume and liquefaction time when using ciprofloxacin, metronidazole and levofloxacin. Although there was non-significant increase in semen pH when using metronidazole and levofloxacin. While there are non-significant decrease in semen pH when use ciprofloxacin at level of ( $P>0.05$ ). There are non-significant increment at level of ( $P>0.05$ ) in sperm motility, non-progressive motility, deoxyribonucleic acid (DNA) fragmentation and white blood cells (WBCs) count in compared between pre and post treatment when using ciprofloxacin, While there are significant increment at level of ( $P<0.05$ ) in normal sperm morphology, round cell count and germ cell count in compared between pre and post treatment when using ciprofloxacin. There are non-significant decrease in the sperm concentration, progressive motility, immotility and agglutination when using ciprofloxacin. The result shows that there are significant decrease in sperm concentration, progressive motility, sperm morphology, round cell count and germ cell count when using metronidazole. The result show that there are significant increase at level of ( $P<0.05$ ) in DNA fragmentation post treatment as compared to pre-treatment. There are non-significant decrease in sperm agglutination and motility percentage post-treatment as compared to pre-treatment. When using metronidazole. The result show that there are non-significant increase at level of ( $P>0.05$ ) in sperm DNA fragmentation and non-progressive motility. While the result show significant decrease at level of ( $P<0.05$ ) in immotile sperm count when using levofloxacin. Although, there are non-significant decrease in sperm agglutination post-treatment in compared to pre-treatment. The results of the effect of antibiotic on round cells was appears that, there is increase in round cell count, germ cell and WBC, progressive motility, motility and concentration when using levofloxacin.*

#### ***Conclusions:***

*From the result of present study appeared to use Endtz test for leukocytospermic men pre-treatment with different drugs was benefit and has*

*accurate and specific result. Administration of antibiotics may be increased sperm DNA fragmentation and count of germ cells in leukocytospermic men. Also, the clinical value of sperm DNA fragmentation detected by acridine orange (AO) is a simple and accurate method.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة:- رويدة فوزي خليل

بإشراف الاستاذ الدكتور أنعم رشيد الصالحي

الاستاذ المساعد الدكتور محمد عودة سلمان

التاريخ: 2013

**Effects of Anti-epileptic Drugs (Phenytoin and Lamotrigine) in Mammalian Model of Embryonic Development**

**Summary**

**Background:**

*The risks associated within utero antiepileptic drugs (AEDs) exposure are of considerable importance. Pregnancies involving maternal health issues other than epilepsy are also at risk for teratogenic AEDs exposure, including neuropathic pain, migraine headaches and psychiatric disorders.*

*Phenytoin is a classical antiepileptic therapy, while lamotrigine is of the new drugs used in this field. This study tries to investigate some aspects of their teratogenic effects.*

*Phenytoin effects on development of the face and in particular development of palatal shelves and palate closure will be considered.*

*Embryonic phase Regarding lamotrigine, the influence of this drug on development of neocortex and hippocampus will be studied, with particular emphasis on neuronal migration in embryonic phase.*

**Aims of the study:**

*This thesis aims to study the effects of:*

*1. Lamotrigine (anti-epileptic drug) on hippocampal development (neural migration) on fetus of the Rats.*

*2. Phenytoin drug on palatal closure on fetus of the Rats.*

**Methods:**

*This study was carried out on rat embryos as a model of mammalian embryo with neural migration of hippocampus and palate similar to that of the human. Three groups, each included (30) pregnant rats, one was a control group, and the second group received 15 mg/kg/ day dose phenytoin IP at*

**VIII**

*E9-E16 of pregnancy whereas the third group was given 20 mg/kg/body weight daily dose lamotrigine IP at E14-E19 of pregnancy.*

*At embryonic day E20, E21 of pregnancy of each group fetuses and litters was retrieved coronal section for head of fetuses and litters made to get palate and hippocampus and were prepared for histological examination to study the effect of both phenytoin on palatal development and lamotrigine on hippocampal development. Respectively for each of group 50 embryos were studied.*

**Result:**

*At E20 and E21, the result showed that there is no effect cell migration and neuronal organization of hippocampal migration in Lamotrigine treated groups.*

*At E20 and E21, the result showed that there is delay union of palatal shelves, there is persist cartilaginous structure is longer than normal in Phenytoin treated group.*

**Conclusion:**

*During the embryonic development of the palatal shelves, there is delay union of palatal shelves but not necessary that is cleft palate it can be seen that the there is persist cartilaginous structure longer than normal in phenytoin treated group during E9-E16 of pregnancy.*

*During the embryonic development of the hippocampus, there is no effect on cell migration and neuronal organization of hippocampal migration in lamotrigine treated groups during E14-E19 of pregnancy.*

*It meant that there was no effect of AEDs especially lamotrigine and phenytoin on stages of development of hippocampus and development of palate*

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الباحثة:- شعوب ياسين حسين

بإشراف الاستاذ الدكتور أنعم رشيد الصالحي

الاستاذ الدكتور محمد باقر محمد رشاد فخر الدين

التاريخ: 2013

**Effect of Sperm Cryopreservation Protocols in Relation to Human Sperm Mitochondrial Apoptosis Activity and Sperm Chromatin Structure**

**Summary**

**Background:**

*Human sperm cryopreservation is a valuable technique in the field of fertility, especially, those patients who are prone to become infertile due to surgical or medical treatments such as chemo- or radiotherapy for cancer treatment. In fact, semen cryostorage seems to be the only proven method that may offer these couples a chance of having children in the future: cancer therapy could in fact lead to damage, resulting in subfertility or sterility due to gonad removal or permanent damage to germ cells caused by adjuvant therapy.*

**Objectives:**

*This study was aimed to investigate the effects of cryopreservation protocols on the human sperm parameters and the effects of cryopreservation on sperm DNA structure and sperm mitochondrial apoptosis.*

**Materials and Methods**

*Sixty semen samples were included in this study. Sperm parameters, DNA fragmentation index (DFI) and mitochondrial apoptosis (MA) were assessed pre- or post-cryopreservation. Cryopreservation was done using either 15% DMSO or 15%*

*glycerol either alone (as control group) or supplemented with either 0.25M or 0.5M of sucrose for treated groups. Crude data were statistically analyzed.*



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الباحث:- وميض رعد عبد الملك

بإشراف الاستاذ المساعد الدكتور انصاف جاسم حسون

الاستاذ المساعد الدكتور محمد عودة سلمان

التاريخ: 2013

## Chronological Determination of Estrogen Receptors Expression in the Testis of Mouse Embryos

### Summary

**Background:** Estrogens are steroid hormones that are very important for both male and female reproductive tract. They are involved in masculine fertility and spermatogenesis. They are important for the development of testes during the embryonic period. However, little is known about estrogen involvement in human testicular organogenesis. Estrogen is synthesized in the male reproductive system and is found in high concentrations in rete testis and seminal fluids. In normal estrogen target tissues, estrogen action is mediated through a specific nuclear estrogen receptors (ER). The site of estrogen action in the developing organism is, therefore, determined by cells that contain ERs. Immunohistochemical methods were used to determine the cellular localization and tissue distribution of ERs in testes of mouse embryos.

**Objectivs of the study:** This study is an attempt to detect the spontaneous serial time, chronology, of ERs expression in mice testicular tissue at prenatal and postnatal life.

**Subjects, Materials and methods:**

Fifty four mature Swiss-Webster mice (*Mus. Musculus*) were used and grouped as follows: 34 mature 8-10 weeks old female mice and 20 postnatal (after birth) mice: 7 newborn (Post partum day 0) males, 7 males 4 weeks old and 6 mature males 8 weeks old.

Three pregnant females were sacrificed every day from 10.5 to 20.5 day post coitum (dpc), and their embryos were collected. From each embryo, one testes was processed for histology and immunohistochemistry (using anti-ER antibody as marker of ERs expression). Before sexual differentiation of mouse embryos at 10.5 and 11.5 dpc the tissue samples of the whole mount embryos were manipulated with

*quick quantitative polymerase chain reaction (q-PCR) technique to determine male gonads by II*

*the detection of specific sex determining region on Y-chromosome (SRY) gene of male embryos.*

*This study is the first of its kind in Iraq at the embryonic level to identifying and measuring the ratio and time of estrogen expression in the male embryos.*

*Results: The first nuclear detection for estrogen receptors was observed in embryonic male gonads at 11.5 dpc. The estrogen receptors expression increased in a linear manner to reach a peak at 17.5 dpc, and continued to increase until a day before birth at 20.5 dpc. The estrogen receptors expression increased further more after birth.*

*Conclusions: Expression of ERs occurred at certain days during mouse embryonic development indicating that the need of estrogen for certain metabolic or morphological events occurring at that time.*

☐ *The first detection of ERs was at 11.5 dpc when the numerous primordial germ cells (PGCs) entered the genital ridges and completed their migration, and there was a rise in ERs expression when colonization of PGCs in the genital ridges was ending at 13.5 dpc.*

☐ *Another rise in ERs expression was at 15.5 dpc during the first descent phase, from the abdomen to the inguinal region.*

☐ *After birth, estrogen played an important role in proliferation and maturation of certain cells in the testes, thus other rises of ERs expression were found.*

☐ *Another rise in ERs expression during puberty in the mature testis when estrogen played an important role in spermatogenesis and sperm maturation.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة:- نور هاني محمد

بإشراف الاستاذ الدكتور سعد صالح الدجيلي

الاستاذ المساعد الدكتور احمد حميد عبود

التاريخ: 2013

Mice Embryonic Development From Cryopreserved Epididymal Sperm Activated by Pentoxifylline and L-carnitine : Experimental Model for Mammals

### Summary

**Background:** It has been the pentoxifylline (PX) and L-carnitine (LC) as motility stimulants showed a positive effect on the activation of sperm in vitro and improve the forward movement after cryopreservation .

**Objective:** The present study aim to investigate the possibility of using PX ,LC and mixture of PX with LC medium for in vitro sperm activation after cryopreservation and its effect in vitro on fertilization rate (FR) and early embryonic development (ED) following in vitro fertilization (IVF) process.

**Materials and Methods:** Fifty mature male and Fifty mature female mice, 8-12 weeks old were used. Epididymal sperms were obtained from caudal region and in vitro direct activation technique was done after cryopreservation by using four media namely; PX alone, LC alone, a mixture of PX with LC and Ham's F-12 (as control media). The four media were used for IVF of mature ova and FR with ED were recorded.

**Results:** In vitro activation of epididymal sperms with PX , LC and mixture of PX with LC has shown positive effect on sperm concentration, sperm motility, and grade activity of progressive forward movement after cryopreservation. There was a significant ( $P<0.05$ ) increase of FR in treated media compared with the control medium after 24 hours of insemination. The study showed that the quality and

*quantity of embryos resulted from in culturing treated media were higher than that of control medium.*

*Conclusion: It is concluded from the results of the present study that adding PX, LC and mixture of PX with LC medium to the sperm activation culture medium and IVF leads to an improvement in certain sperm function parameters, supports the FR and early ED rate.*

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رسالة الماجستير في علم الاجنة التطبيقي

الباحثة:- مها فوزي جلاب

بإشراف الاستاذ الدكتور أنعم رشيد الصالحي

الاستاذ المساعد الدكتورة نوال خيري حسين

التاريخ: 2013

Morphometrical study of the effects of nicotine on the lung of rat fetuses and offspring

### Summary

*Background : Smoking during pregnancy remains common , and harms both the mother and her developing fetus. Maternal smoking during pregnancy has long been considered an important risk factor for intrauterine growth retardation. The umbilical cord is the developing fetus life line, the blood that flows through this cord gives the fetus all the oxygen (O<sub>2</sub>) and nutrients it needs to grow, When a mother smokes a cigarette, she inhale the gas carbon monoxide. This means that the amount of oxygen available to her fetus through the umbilical cord is reduced. This makes the fetus heart beat more rapidly, and increases overall stress on its developing body . Nicotine is one of cigarette smoking components, it can reduce the flow of blood through the placenta, which limits the amount of nutrients feeding the fetus and thus affecting multiple organs in the fetus and newborn ,and the body growth generally. The lung is one of these organs affected by nicotine that developed prenatally in stages that continue on after birth .*

*Objectives of the study: This study is an attempt to detect the effect of nicotine on body weight , lung weight and ratio between them in rats fetuses and offspring as well as to investigate the changes that occur in morphometric of the developing lungs .*

*Materials and methods: Seventy mature healthy female rat were used in the current study. They were divided in to two groups, each included (35) pregnant rat,*

*the first group received normal saline subcutaneous during gestation and lactation period and considered as control, the second group received (1 mg/kg body weight daily) dose nicotine subcutaneous, during experimental period that extend from the first day of gestation and complete to week after parturition and considered as experimental group.*

*Lungs from fetuses of developmental stages in E16, E19, E20 was retrieved and the offspring in day one and day seven after parturition was prepared for histological examination.*

*Morphometric evaluation of the lungs was performed using Motic image plus version 2.0 image analysis system. Morphometric parameters included area and perimeter of alveoli in developing lung.*

*Results: The present study showed that nicotine caused a significant reduction ( $P < 0.05$ ) in the total body weight and in lungs weight of fetuses and offspring, while the effect of nicotine on the lung/body weights ratio has no significant difference ( $P > 0.05$ ) in fetuses and significant difference ( $P < 0.05$ ) in offspring.*

*Histomorphometric measurements of area and perimeter of developing alveoli were increased significantly ( $P < 0.05$ ) compared with the control group in all ages except that in day one after parturition was non significant difference ( $P > 0.05$ ).*

*Conclusion: From the results of the current study, it was concluded that after nicotine injection to pregnant rats there was decrease in body weight and lung weight and alteration in morphometric of lungs in the fetuses and offspring.*