Journal of College of Education for Pure Sciences
Volume 8, Number 2, June 2018
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

Study Effects of Oral Contraceptive on Some hormones hematological Parameters and Testis of Experimental Male Rat

Shaymaa Z. Al-Rumaidh[1] Sabah Hussain Enayah[1,3] Bidaa Ali[2]

(1) Department of biology, College of Science, University of Thi-Qar ,Thi-Qar , Iraq

(2) Department of Pathology Analysis, College of Science, University of Thi-Qar ,Thi-Qar , Iraq.

(3) Toxicology program, Graduate collage, University of Iowa, USA.

Abstract

Oral contraceptive, Birth control, pills are mainly used to prevent the pregnancy. The combination of progesterone and estrogen are commonly use in these days. In this paper, we aimed to determine the influence of progesterone and estrogen as synthetic chemical found in oral contraceptive pills on male rats. we investigated the contribution of oral contraceptive on 2 groups of male rats. 25 and 50mg/kg a had been given through gavage for 60 days. Level of testosteronealso were measured utilizing Minvids. Determination of red blood cells, RBCs, total white blood cells and platelets PLwhere been counted using coulter counter. There was a significant influence of oral contraceptive pills on WBC and PLT while there are not significant effect of RBC comparing to the control. Moreover, in the current study level of Testosterone was not significantly affected due to treatments comparing to the control in both groups. Eosin and Hematoxylin stain used to make histological sections of testiest and the results showed that normal spermatogenesis can be seen clearly in the control group after 60 days' exposure. The spermatogenesis is disrupted based on dose where 50mg of oral contraceptive exposure showed very high level of disturbance. Reductions in the normal designand abnormal distance in seminiferous tubules also noticed after 60 day's treatment with oral contraceptive. In addition to reduce the number of germ cells and somatic cells population after 50mg exposure are counted in high level of contraceptive taken.

مجلة كلية التربية للعلوم الصرفة - جامعة ذي قار
المجلد ٨، العدد ٢، حزيران ٢٠١٨
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

Study Effects of Oral Contraceptive on Some hormones hematological Parameters and Testis of Experimental Male Rat

Shaymaa Z. Al-Rumaidh[1] Sabah Hussain Enayah[1,3] Bidaa Ali[2]

(1) Department of biology, College of Science, University of Thi-Qar ,Thi-Qar , Iraq

(2) Department of Pathology Analysis, College of Science, University of Thi-Qar ,Thi-Qar , Iraq.

(3) Toxicology program, Graduate collage, University of Iowa, USA.

الخلاصة

تهدف الدراسةالحالية الى لمعرفه التغيرات الكيموحيويه لحبوب مانع حمل التي جرعت فمويا لذكور١٨ من الجرذان لمده ٦٠ يوما. حيت تضمنت الدراسة تركيزين مختلفين لعقار مانع حمل (25 ,50 mg/kg). عند انتهاء مده الدراسة جمعت عينات دم من الجرذان لتشخيص كل من هرمون التستيرون كريات الدم الحمراء وكريات الدم البيضاءوالصفائحالدموية. اظهرت نتائجالدراسةالحالية عدم تغير في تركيز هرمون التستستيرون وانخفاض معنوي في كريات الدم البيض عند مستوى معنوي $P\leq0.05$. كما اكدت الدراسة عدم تأثر كريات الدم الحمر وارتفاع مستوى الصفائح الدموية عند نفس المستوى المعنوي. اضافه الى ما تم ذكره سابقا فقد تضمنت الدراسة اجراء مقاطع نسيجيه لخصى ذكور الجرذان المعاملة بحبوب مانع حمل. أوضحت نتائج المقاطع النسيجية ان العلاج المستخدم كان قادر على اجراء بعض التغيرات النسيجية التي اشتملت تفكك النسيج الخلوي للخصية وتحلل النسج المحيط للأنابيبالمنوية وتلف كبير في خلايا لايدك كما تبين ان مانع الحمل قد خفض العدد الكلي للنطف بشكل ملحوظ. لذا فان العقار قد ترك اثر واضح على الخصوبة لما سببه من تلف نسجي وانخفاض عدد النطف.

Introduction

The world's population continue to increase by 80 million yearly. The expectation the population will reach 10 billion in 2050 [1, 2]. Increase number of population would lead to environmental degradation and human suffering from deficiency in supplies[3].Abundant number of this population, in fact, is accidentally happened. In 2008, Specifically, 41% of the pregnant women were unprepared to that and this lead to abortion of 20% from the pregnancies worldwide [4, 5]. The elevated frequency of unintended pregnancy isbecause the women are a terrifiedfrom the side effects or there are no excess to contraceptive [2]. 98% of these women would trust their partner to use some sorts of contraception [6]. Several of surveys demonstrated that male contraceptive analogous to the combination of estrogen and progesterone pillswould be welcomed by much of men worldwide [7, 8].

Oral hormonal contraceptive pill, in drug regulatory classification, is represented as drug which have been taken by health people rather than by men who suffer from certain disease. Consequently, effective contraceptive carry no health risks or side effect are the major concern about oral contraceptive pills in men [9-11].

Several analyses about hormonal male contraceptive showed that there isan important perspective of men which are taken more seriously by enormous number of experts and policymakers[12]. Most of them concern about men's emotional wellbeing and sexuality. Men's happiness has been emphasized and put on the international research agenda by scientists and pharmaceutical industry[12, 13].

Nevertheless, over last three decades, reports on clinical trials suggest an increasing number of side effects that are associated with male hormonal contraceptive. The evaluation of health risks of male taken hormonal contraceptive exemplified by the effects of hormones on lipids which become as major indicator in this case[14].

Women effected by contraceptive has been the central focus of birth control delegation. Based on our knowledge there are no many studies considered the impact of oral contraceptive pills, which has been commonly taken by female, on male health. Thus, in the present study, the authors tried to investigate the role of oral contraceptive pills on some blood figures and male hormone.Histological section for testis and sperm counts also elucidated in this study. The big picture of the current study is to find out the real influence of hormonal contraceptive on male and if the percentages of progesterone and estrogen combination in the third generation of this medicine which derived from the synthetic testosterone.

Materials and methods

Wister albino rate species at age (10-12) weeks and weight (150 to 250 g) had been used to this experiment. All the animals gained and kept at the animal's house facilities of biology department in College of Science at Thi-Qar university. Plastic cages and standard condition had proposed in this experiment. Animals in all groups were given free access to food and water. After animals had been divided to three groups each group contain 6 male rats, the animals left about 10 days for adaptation.

Contraceptive pills that commercially available were crushed and dissolved in tap water. The dosage used calculated based on women usage level. The oral gavagetechnique was the main method to deliver the normal saline or the mixture of oral contraceptive in the current experiment.

The groups included; control group where normal saline had been given on daily base for 60 days. Second group were daily administrated to 25mg per body weight of oral contraceptive. Third groupwere demonstrated to 50mg per body weight of oral contraceptive.

At the end of the experiment, animals were sacrificed afteranesthetized with chloroform as descript in[15, 16]. blood samples were collected through cardiac puncture. Blood divided into 2 kinds of tubes; tube with EDTA for hematology analysis and tube without EDTA for serum collection and hormone measurements.

EDTA tubes used for red blood cell (RBC), white blood cells (WBC) and platelet (PL) using coulter counter in Thi-Qarhospital. Hormone analysis conducted using Minivids also with assistance from Thi-Qar university. The testis was dissected and fat tissue was removed.

The testis was harvested and fix in 10% paraffin.Slides of each rat's testis were prepared and H& E stains utilized as described in [17]. In blinded manner, 4 slides from each treatment and 10 round of seminiferous tubules(ST) of each were selected randomly to count number of germ cells (spermatogenesis evaluation). Spermatogonia type A and B for testicular cells quantifiedusinglight microscope Olympus with lenses 40X. Johnson Scoring system was been used for rating germ cells maturation in each ST using score of 1-10[18, 19]. The counting repeated twice with two different specialists.

Statistical analysis

The data which has been shown in this study calculated in SAS 9.3 or GraphPad prism 6. The statistics different between control and treated groups were analyzed using tow way ANOVA followed by Bonferroni.A p-value of <0.05

Results

Level of Testosterone (T)was low in treatment groups but is not reduced enough to be significant comparing to the control. The average of testosterone in animals treated with 50mg/kg (0.3±0.04) and group treated with 25mg/ kg was(0.25±0.15) decreased slightly comparing to control (4.7±0.4) as showed in figure (1).

Regarding to the hematology parameters which included in this study, the results showed that level of white blood cells (WBC) significantly reduced in the end of the experiment in 25 mg treated group (9.83±1.22) and 50mg treated group (6.07±1.36) comparing to the control Figure (2). Figure (3) illustrated red blood cell count RBC. RBC level dose not changed after 60 day orally exposed animals to hormonal combination of contraceptive with 25mg (5.63±0.59) and the case was same with higher concentration (5.13±0.59).

Testis histological observation showed that seminiferous tubules (ST) incontrol group were compactly arranged. Their shapes were mostly oval or semi-around with intact basement membrane. The average of germ cells was normal in control sections. Masses of spermatozoa can be seen in control sections Figure (5A). stages of spermatogenesis can be seen in most of seminiferous tubules. Section of testes from rats treated with 25mg of oral contraceptive for 60 days showed marked deteriorating changes, while the seminiferous tubules appeared as same size. The basement membrane intact whereas the germ cells were extremely condensed. Number of somatic cells are less and necrotic cells showed as fragments in figure (5B). Noticeable degenerative alternation showed in section of testis from animals exposed to 50mg of oral contraceptivesuch as decrease in the normal architecture and abnormal distance in seminiferous tubules. More reduction in the germ cells and somatic cells population wasobserved. At this dose, atrophy of seminiferous shape and basement membrane with alteration in lumen also appeared. Mean of spermatogonia type A and B countsof treated groups and control male were as following; control 9.4±0.02, 25mgoral contraceptive treatment 8.83±0.14 and 25gm treatment 6.83±0.11.

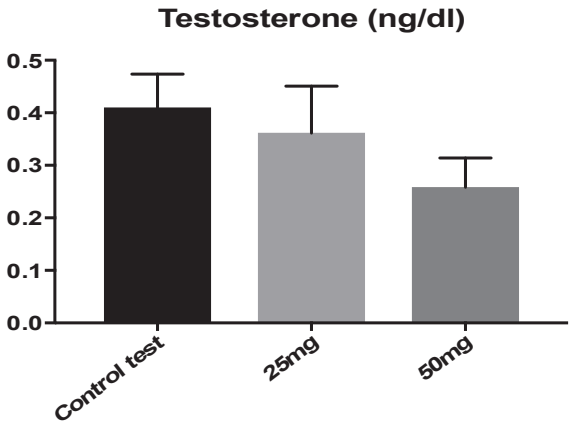


Figure 2: level of Testosterone hormone in male rate after 60 days' treatment with oral contraceptive. no significant change showed at the end of the experiment P≤0.05.

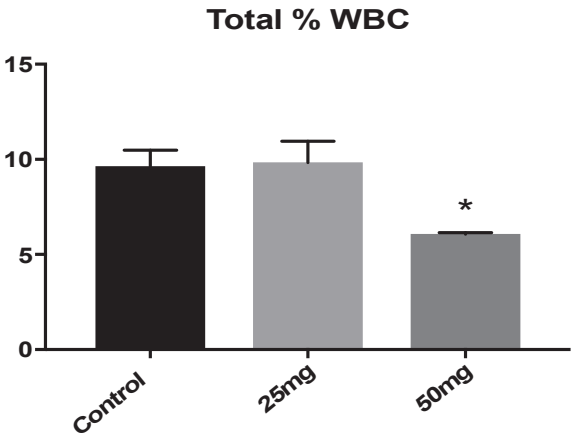
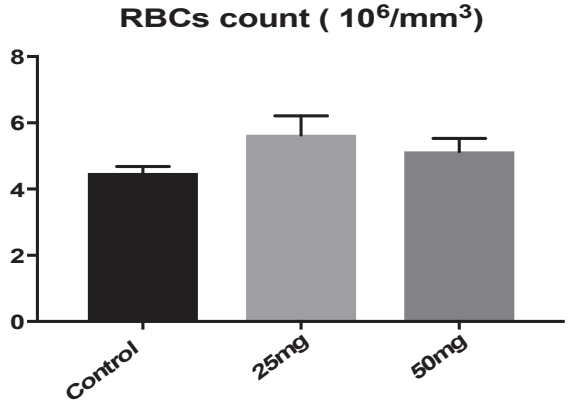


Figure 3: WBC after 60 days' treatment with tow concentrations of hormonal oral contraceptive to male rate. At high concentration WBC went significantly down comparing to the control P≤0.05.



Figu**re 4**: level of RBC at the end of the experiment showing there are no significant different in both treatment groups comparing with control P≤0.05.

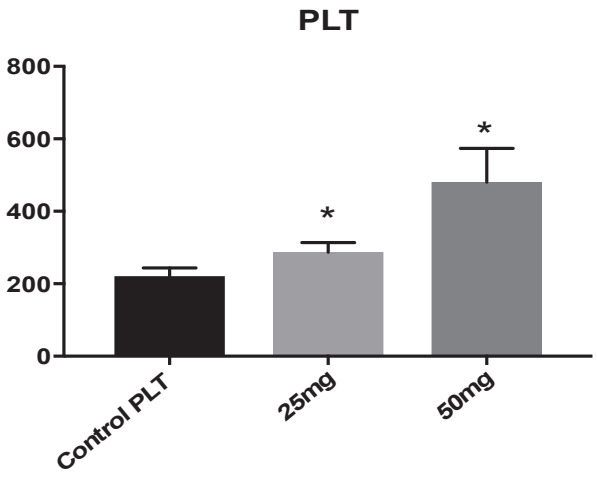


Figure 5: Platelet level in male rate treated with oral contraceptive showed significant different with both treatment groups. P≤0.05

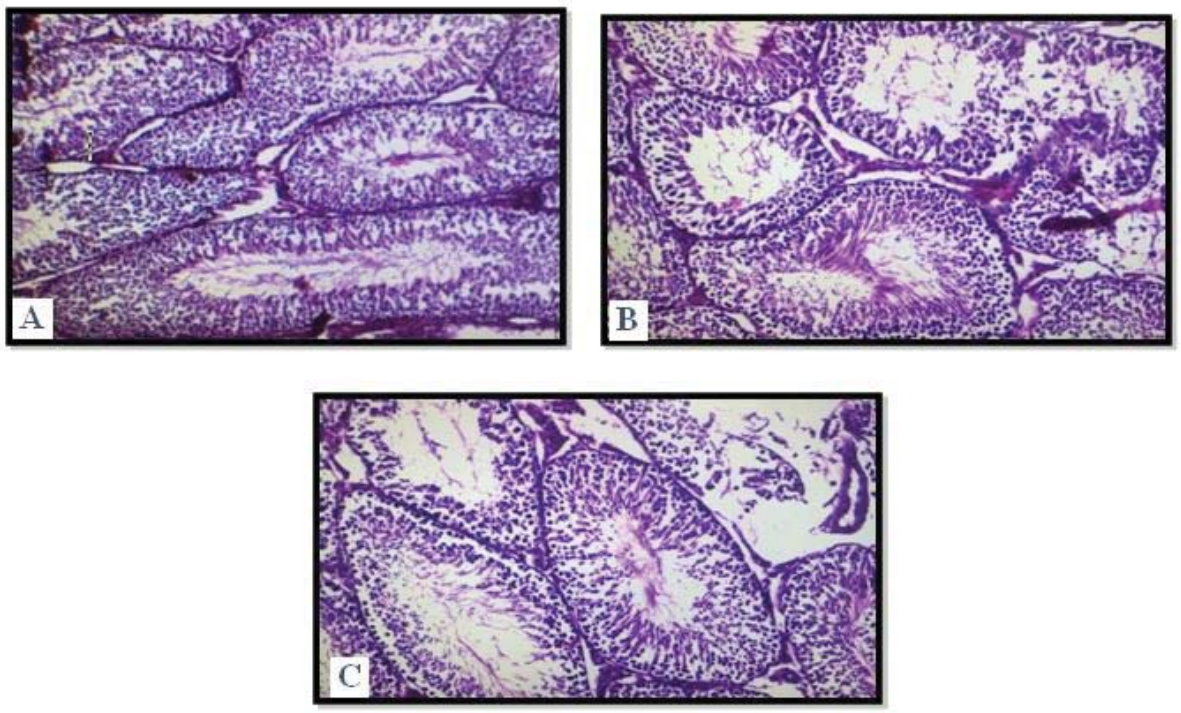


Figure 6: Histological sections of rat testes stained with H&E. Control group and normal spermatogenesis can be seen (wide raw) (A). After 60 days' exposure to 25mg of oral contraceptive stages of spermatogenesis (B). Reductions in the normal design (wide raw) and abnormal distance in seminiferous tubules (head raw). In addition to reduce the number of germ cells and somatic cells population after 50mg exposure(thin raw) (C).

Discussion

Contraceptive pills mostly are combination of estrogen and progesterone[11]. The main mechanism of action is the inhibition of follicular development and prevent ovulation. Negative feedback of progestogen in hypothalamus would decrease the pules frequency resealing of gonadotropin hormone. The reduction of gonadotropin will reduce the secretion of follicle stimulating hormone(FSH) and would also drop luteinizing hormone (LH). Inhibit follicle development will reduce the estradiol levels[20, 21].

In contrast, the main mechanism of action to combined oral contraceptive pills is progesterone's ability to impede sperm form penetrating through the cervix and upper genital tract[22, 23]. In addition, thereduction in level of LH and FSH from the pituitary, in turn, will not stimulate leyding cell to produce testosterone(T) and sertoli cell to endorse spermatogenesis[24].

Based on information from above we should see decrease in T level but This came out differently with what have been proven in the current study. The reduction in level of T hormone was not be enough to be significant because the duration of experiment probably was not long enough to show such differed. The result of our study agreed with pilot study where sixteen volunteerswastaken oral progestin. This study demonstrated that sperms count will be at very low values after three months of treatment. Level of LH and FSH among these men were inhibited while plasma testosterone average was remained within equal range[25, 26].

Over decades, most of the scientists and physician are using WBC as one of the frequently ordered tests in the clinical medicine. There are no many of noninfectious factors that could alter WBC value. hormonal oral contraceptive has been known to change blood parameters. A study on14,961 healthy women who was taking daily oral contraceptive were evaluated to determine the role of contraceptive in changing WBC. The results of this study showed that the level of WBC statically increased among the women who was continue taking the pills over three months [27]. Another study about of 38 students showed that the students, who are exposed to difficult academic examination and taken oral contraceptive, demonstrated a significant positive relationship increase in WBC comparing to student who were just introduced to tough examination [28]. Moreover, a study aimed to investigate the factors which increase's inflammation and consequently increase WBCamong adolescents and young women and explore if these factors would be effected by oral contraceptive. The result confirmed that WBC would be increased with women taken oral contraceptive comparing with who are not [29]. The present finding is agreed with all the previous results showed above. The level of WBC's illustrates statically increase with both concentrations.

Diminish absorption of dietary folate and polygutamic are well known as side effects of oral contraceptive uses. A study included 3736 women were RBC parameter analyzed. Oral contraceptive users displayed evaluation in the level of RBC [30]. Another epidemiological study showed that there is no effect of oral contraceptive on hemoglobin, hematocrit and erythrocyte count[31-33]. The outcome to the present study aligns well with those of former studies where no significant change had been shown in the RBC. Due to the Confounding of the effects of dose and the type of hormone and the formulation of the drug, it was not easy to estimate the effect of oral contraceptive on RBC.

Even though, the combination of estrogen and progesterone would be considered as advance formal of the oral contraceptive, the use of it still strongly associated with vascular disease [34, 35]. Increased coagulation activity ismostly dependent on estrogen dose. Several works suggested that the upsurge of clotting activity derived from oral contraceptive uses is mainly resulted from platelets increase[36]. It also has reported that oral contraceptive would increase collagen and Thromboxane A2TXA$_2$ which consider the main reason for platelet aggregation increase. Studies dealing with women and experimental animals reported that using oral contraceptive caused platelet hyperactivity[37]. Our finding of oral contraceptive enhances of rise platelet count in both doses comparing with control that would agree with finding of epidemiological studies cited previously.

Alternation in testiest histology with the dose of treatment that been represented by decrease germ cells and atrophy of seminiferous tubules is due to the elevation of the FSH or and estrogen level[38, 39].Taking oral contraceptive would increase level of exogenous FSH and estrogen and the dominance of these hormones in male rats impede spermatogenesis due to alteration in balance of male hormone and inhibit receptors for male hormones.Due to powered androgen the male system would be crashed and elevated of germ cell necrosis[40].

Accordingto reviewed previous studies, there are no studies elucidate wither the female hormonal contraceptive would affect on the blood parameters as well as hormones and histology of testiest. The current study clearly showed evidence that female oral contraceptive which might been taken by male would have serious adverse effect on blood parameters. In the same time these pillscould impact on testicular structure in both morphology and sperm production that in turn may lead to poor fertility specially at high level.

Acknowledgments

The present study was supported by biology department in collage of Science Thi-Qar university. We deeply thank Dr. Khalid al fartosi for the animals providing. The authors thankful for Dr. MhaSharker the dean of pharmacy college for her assistance with slides reading.

References:

.١ Kopf GS: Approaches to the identification of new nonhormonal targets for male contraception. Contraception 2008, 78(4 Suppl):S18-22.

.٢ Nya-Ngatchou JJ, Amory JK: New approaches to male non-hormonal contraception. Contraception 2013, 87(3):296-299.

.٣ Speidel JJ, Grossman RA: Addressing global health, economic, and environmental problems through family planning. ObstetGynecol2011, 117(6):1394-1398.

.٤ Singh S, Sedgh G, Hussain R: Unintended pregnancy: worldwide levels, trends, and outcomes. Studies in family planning 2010, 41(4):241-250.

.٥ Shah I, Ahman E: Unsafe abortion in 2008: global and regional levels and trends. Reproductive health matters 2010, 18(36):90-101.

.٦ Glasier AF, Anakwe R, Everington D, Martin CW, van der Spuy Z, Cheng L, Ho PC, Anderson RA: Would women trust their partners to use a male pill? Hum Reprod2000, 15(3):646-649.

.٧ Martin CW, Anderson RA, Cheng L, Ho PC, van der Spuy Z, Smith KB, Glasier AF, Everington D, Baird DT: Potential impact of hormonal male contraception: cross-cultural implications for development of novel preparations. Hum Reprod2000, 15(3):637-645.

.٨ Heinemann K, Saad F, Wiesemes M, White S, Heinemann L: Attitudes toward male fertility control: results of a multinational survey on four continents. Hum Reprod2005, 20(2):549-556.

.٩ HasbySaad MA, Radi DA, Hasby EA: Oral contraceptive pills: Risky or protective in case of Trichinella spiralis infection? Parasite Immunol2017, 39(8.(

.١٠ Naz F, Jyoti S, Rahul, Akhtar N, Siddique YH: Effect of Oral Contraceptive Pills on the Blood Serum Enzymes and DNA Damage in Lymphocytes Among Users. Indian J ClinBiochem2016, 31(3):294-301.

.١١ Cooper D, Adigun R: Oral Contraceptive Pills. In: StatPearls.edn. Treasure Island (FL); 2017.

.١٢ Rowen TS: Treating Sexual Side Effects from Oral Contraceptive Pills. J Womens Health (Larchmt) 2017, 26(7):711.

.١٣ Kanakis GA, Goulis DG: Male contraception: a clinically-oriented review. Hormones (Athens) 2015, 14(4):598-614.

.١٤ Arowojolu AO, Gallo MF, Lopez LM, Grimes DA: Combined oral contraceptive pills for treatment of acne. Cochrane Database Syst Rev 2012(7):CD004425.

.١٥ Knez J: Endocrine-disrupting chemicals and male reproductive health. Reproductive BioMedicine Online, 26(5):440-448.

.١٦ Ahmed SI, Ali TO, Elsheikh AS: Ultra-structure of testes of rats born to dams treated with hydroxy-progesterone hexanoate. Asian Pacific Journal of Reproduction 2016, 5(6):510-513.

.١٧ Batra N, Nehru B, Bansal MP: Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. Journal of Applied Toxicology 2001, 21(6):507-512.

.١٨ Rosai J: Controversies in surgical pathology. Int J SurgPathol2004, 12(4):299-300.

.١٩ Johnsen SG: Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormone Research in Paediatrics1970, 1(1):2-25.

.٢٠ al-Mizyen E, Sabatini L, Lower AM, Wilson CM, al-Shawaf T, Grudzinskas JG: Does pretreatment with progestogen or oral contraceptive pills in low responders followed by the GnRHa flare protocol improve the outcome of IVF-ET? J Assist Reprod Genet 2000, 17(3):140-146.

.٢١ Curtis KM, Chrisman CE, Mohllajee AP, Peterson HB: Effective use of hormonal contraceptives: Part I: Combined oral contraceptive pills. Contraception 2006, 73(2):115-124.

.٢٢ Damario MA: Ovarian hyperstimulation syndrome prevention strategies: oral contraceptive pills-dual gonadotropin-releasing hormone agonist suppression with step-down gonadotropin protocols. SeminReprod Med 2010, 28(6):468-474.

.٢٣ DastranjTabrizi A, MostafaGharabaghi P, SheikhzadehHesari F, Sadeghi L, Zamanvandi S, Sarbakhsh P, Ghojazadeh M: Impact and mechanistic role of oral contraceptive pills on the number and epithelial type of ovarian cortical inclusion cysts; a clinicopathology and immunohistochemical study. DiagnPathol2016, 11:30.

.٢٤ Dinger J, Minh TD, Buttmann N, Bardenheuer K: Effectiveness of oral contraceptive pills in a large U.S. cohort comparing progestogen and regimen. ObstetGynecol2011, 117(1):33-40.

.٢٥ Guerin JF, Rollet J: Inhibition of spermatogenesis in men using various combinations of oral progestagens and percutaneous or oral androgens. International journal of andrology 1988, 11(3):187-199.

.٢٦ Soufir JC, Jouannet P, Marson J, Soumah A: Reversible inhibition of sperm production and gonadotrophin secretion in men following combined oral medroxyprogesterone acetate and percutaneous testosterone treatment. Actaendocrinologica1983, 102(4):625-632.

.٢٧ Fisch IR, Freedman SH: Smoking, oral contraceptives, and obesity. Effects on white blood cell count. Jama 1975, 234(5):500-506.

.٢٨ Maes M, Van Bockstaele DR, Gastel A, Song C, Schotte C, Neels H, DeMeester I, Scharpe S, Janca A: The effects of psychological stress on leukocyte subset distribution in humans: evidence of immune activation. Neuropsychobiology1999, 39(1):1-9.

.٢٩ Ibanez L, Jaramillo AM, Ferrer A, de Zegher F: High neutrophil count in girls and women with hyperinsulinaemichyperandrogenism: normalization with metformin and flutamide overcomes the aggravation by oral contraception. Hum Reprod2005, 20(9):2457-2462.

.٣٠ Fisch IR, Freedman SH: Oral contraceptives and the red blood cell. Clinical pharmacology and therapeutics 1973, 14(2):245-249.

.٣١ Prasad AS, Oberleas D, Moghissi KS, Lei KY, Stryker JC: Effect of oral contraceptive agents on nutrients: I. Minerals. Am J ClinNutr1975, 28(4):377-384.

.٣٢ Larsson G, Milsom L, Lindstedt G, Rybo G: The influence of a low-dose combined oral contraceptive on menstrual blood loss and iron status. Contraception 1992, 46(4):327-334.

.٣٣ Jamil S, Khan RA, Dilshad H, Fatima S: Haematologic Variations Associated With the Long Term Use of Contraceptives in Young Females. International Journal of Medical Research and Review 2014, 2(5):580-586.

.٣٤ Durand P, Blache D: Enhanced platelet thromboxane synthesis and reduced macrophage-dependent fibrinolytic activity related to oxidative stress in oral contraceptive-treated female rats. Atherosclerosis 1996, 121(2):205-216.

.٣٥ Vessey MP, Villard-Mackintosh L, McPherson K, Yeates D: Mortality among oral contraceptive users: 20 year follow up of women in a cohort study. Bmj1989, 299(6714):1487-1491.

.٣٦ Norris LA, Bonnar J: Effect of oestrogen dose on whole blood platelet activation in women taking new low dose oral contraceptives. Thrombosis and haemostasis1994, 72(6):926-930.

.٣٧ Roell A, Schueller P, Schultz A, Losel R, Wehling M, Christ M, Feuring M: Effect of oral contraceptives and ovarian cycle on platelet function. Platelets 2007, 18(2):165-170.

.٣٨ Gopalkrishnan K, Gill-Sharma M, Balasinor N, Padwal V, D'Souza S, Parte P, Jayaraman S, Juneja H: Tamoxifen-induced light and electron microscopic changes in the rat testicular morphology and serum hormonal profile of reproductive hormones. Contraception 1998, 57(4):261-269.

.٣٩ Mok K-W, Mruk DD, Lie PP, Lui W-Y, Cheng CY: Adjudin, a potential male contraceptive, exerts its effects locally in the seminiferous epithelium of mammalian testes. Reproduction 2011, 141(5):571-580.

.٤٠ Walker WH: Non-classical actions of testosterone and spermatogenesis. Philosophical Transactions of the Royal Society of London B: Biological Sciences 2010, 365(1546):1557-1569.