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Ovarian Histopathological Lesions by Superovulation Induction Drugs Intake in Female Rats

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Abstract. This study was conducted to determination the effects of fertility drugs on some sex hormones and the histological changes of the ovary. Forty-two of female rats were used, it divided into sevsn groups, each group containing 6 rats. The first group was fed with distilled water and feed as control group. The second and third groups were received Clomid (50 mg) for one month and two months respectively. The fourth and fifth groups were given Duphaston (10 mg) for one month and two months respectively. The sixth and seventh groups were treated with the Procreation V for one and two months. The results showed a singnificant increasing in levels of sex hormones (estrogen, progesterone and prolactin) in addition to histological damage such as the decline of ovarian follicles with congestion, edema, fibrosis, bleeding, increasing thickness of germinal layers and inflammation, as it was found that exposure to them for a period of two months is the most harmful.

Highlights:

1. Drugs increased estrogen, progesterone, and prolactin levels significantly.
2. Histological damage: follicle loss, inflammation, fibrosis, bleeding.
3. Two-month exposure caused the most severe ovarian harm.

Keywords: Fertility drugs, ovary, progesterone, histological lesions

Introduction

The ability to reproduce is crucial for the long-term survival of humanity. However, infertility can arise from various issues affecting both males and females. Several factors contribute to infertility in women, including sexually transmitted diseases such as gonorrhea and syphilis. Additionally, obesity, smoking, alcohol consumption and conditions like diabetes ,hypertension and conditions like diabetes, hypertension, and hypothyroidism have also been linked to an increased prevalence of infertility[1].Other causes of infertility include factors such as ovulation disorders fallopian tube abnormalities endometrial issues and other contributing factors [2].Fertility drugs are medications that work to promote fertility levels in male and female and are considered one of the treatment options used to enhance and treat fertility [3]. These medications

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contain minerals, vitamins, herbs, and other amino acids that effect on the reproductive system , It help enhance reproductive fertility in female by stimulation the growth of ovarian follicles, Clomiphene citrate (CC) is one of the most common of these medications and has the ability to interact with tissues that contain estrogen receptors, including the hypothalamus, ovary, pituitary gland, endometrium, cervix, and vagina , It may compete with estrogen for estrogen receptor binding sites and may delay the regeneration of estrogen receptors within cells [4]. Women with anovulatory infertility are treated with clomiphene citrate, a selective estrogen receptor modulator that blocks the negative feedback of endogenous estrogen on the hypothalamic-pituitary axis, leading to increased FSH secretion and ovulation [5]. In addition, follicular development, ovarian enlargement, nausea, vasomotor flashes, vomiting, headache, breast pain, visual signs, excessive vaginal bleeding, and weight gain are all side effects of clomiphene citrate [6]. However, it was later shown that this treatment has many harmful effects, including ovarian and uterine abnormalities [7].

Objective of the study

The aim of the present study was evaluation in sex hormones and histological damages the changes in the structure and function of ovary by infertility drugs intake.

Methods

Fertility stimulants (infertility drugs)

Three types of infertility drugs were used in the current study, namely Clomiphene citrate (50mg) / France, Procreation V (500mg) / USA, Duphaston (10mg) / Netherlands.

Animals

Female laboratory rats weighing (215-225) grams and aged (11-13) weeks were used 42. These animals were obtained from the animal house at Thi-Qar University, College of Education for Pure Sciences. Under laboratory conditions (20±3 ^{O}C) and (12h) light and (12h) darkness.

Experimental Design

The laboratory rats were divided into seven groups, each group included six rats as follows:

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Group 1: These animals were given the normal feed and distilled water as control group.

Group 2: The animals were treated with Clomid (50mg) for one month

Group 3: The animals were given Clomid (50mg) for two months.

Group 4: The animals were given Duphaston (10mg) for one month.

Group 5: The animals were given Duphaston (10mg) for two months.

Group 6: The animals were given Procreation v (500mg) for one month.

Group 7: The animals were given Procreation v (500mg) for two months.

All of above medications were taken orally by a stomach tube.

Sex Hormones Measurement

Sex hormones measurement was involved estrogen, progesterone and prolactin. Blood samples were centrifuged at 2500 rpm for 15 minutes for sera obtaining which it used later for assessment these hormones by ELF technique with Mini VIDAS device.

Histological Examination

Ovaries of all groups were eradicated for histological preparation, it were fixed by formalin (10%) then dehydrated by ascending concentrations of ethyl alcohol.Xylen was used for clearing , after that it were embedded in paraffin wax and sections with 5 microns were stained by hematoxylin and eosin .

Statistical Analysis

Data were analyzed by Spss (version 25). Significance was calculated at ($p \leq 0.05$) when comparing among groups [8].

Result and Discussion

Biochemical results

The results of the current study showed a significant increase ($P \leq 0.05$) in the levels of sex hormones (estrogen, progesterone and prolactin) in all female animals treated with fertility stimulants when compared with the control group except procreation V group which was estrogen non-significant increased compared with control group, also progesterone was non-significant increased compared with all groups that treated with all drugs for one month compared with control.

The groups treated with clomid and duphaston for two months showed a significant increase ($P \leq 0.05$) in the levels of these hormones when compared with the

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groups treated with Clomid and Duphaston for one month. While the increase in prolactin and progesterone did not reach the level of significance level in the groups treated with the Procreation v for two months when compared with groups that treated with the Procreation v for one month, while the estrogen hormone increased significantly (P≤0.05) for the group treated with the Procreation v for two months when compared with the group treated with the same supplement for one month.

When comparing all the groups treated with fertility stimulants with each other, it was found that the highest values of these hormones were Clomid two months group while the lowest rate was Procreation v for one month group.

Table (1) shows the effect of fertility stimulants on the level of sex hormones in female (mean ±S. D)

Groups	Progesterone ng / ml) (	Estrogen ng / ml) (	Prolactin ng / ml) (
Control group	22.94±0.83^{d}	204.79±0.96^{e}	3.27±0.35^{e}
Clomid group for one month	23.54±0.59^{cd}	219.16±1.27^{c}	4.29±0.16^{c}
Clomid group for two months	25.81±0.32^{a}	232.31±2.69^{a}	5.92±0.15^{a}
Duphaston group for one month	23.23±0.72^{cd}	218.29±2.73^{c}	4.07±0.08^{cd}
Duphaston group for two months	24.73±0.54^{b}	227.86±2.40^{b}	5.25±0.37^{b}
Procreation v group for one month	23.24±1.07^{cd}	207.56±6.63^{e}	3.81±0.14^{d}
Procreation v group for two months	23.86±0.49^{c}	213.15±2.17^{d}	3.89±0.49^{d}

- Different letters indicate to differences significantly (P≤ 0.05)

Histological Results

Histological examination of ovary for control group was pointed to normal structural and number of ovarian follicles (figure 1) It was noted that taking fertility stimulants drugs such as clomid and duphaston for two exposure periods (1 month - 2 months) caused comprehensive destruction of tissue and congestion and necrosis in

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multiple locations within the ovary with the appearance of fibrosis and bleeding, in addition to a significant increase in the thickness of the germinal layer surrounding the ovarian follicle, also, vacuolation and decline in number of ovarian follicles and inflammatory infiltration. While the normal structure of the ovarian tissue was appeared with congestion and slight rupture in the groups which treated with procreation V for the same two exposure periods.

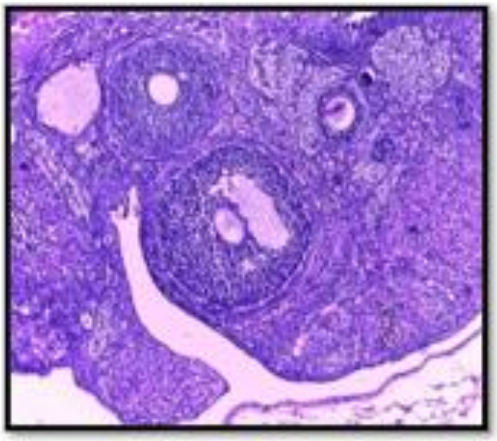

Figure (1): Cross section of the ovary in the (control group) showed ovarian follicles (A) Capsul(B)(H&E)(100X).

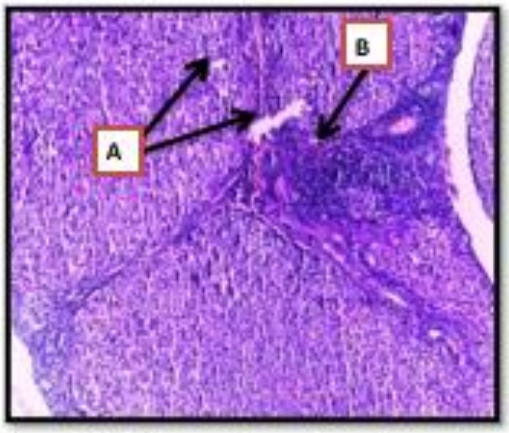

Figure (2): Cross section of ovary in group (Clomid 1 month) showed necrosis (A) with infiltration (B) (H&E)(100X).

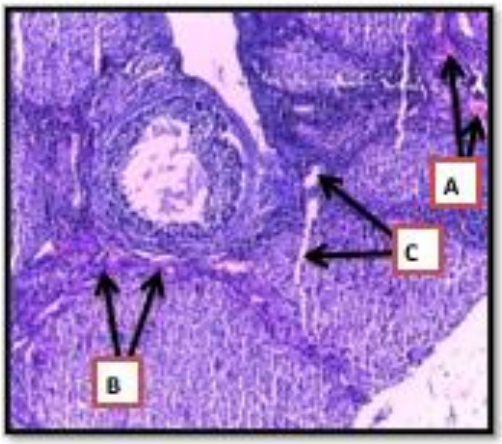

Figure (3): Cross section of ovary in group (Clomid 1 month group) showed Congestion (A) fibrosis (B) necrosis (C) (H&E)(100X).

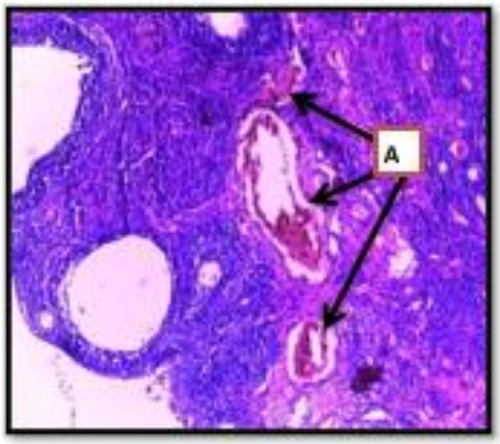

Figure (4): Cross section of the ovary in the (Clomid 2 months group) showing severe Congestion in multiple locations within the tissue (A) (H&E) (100X)

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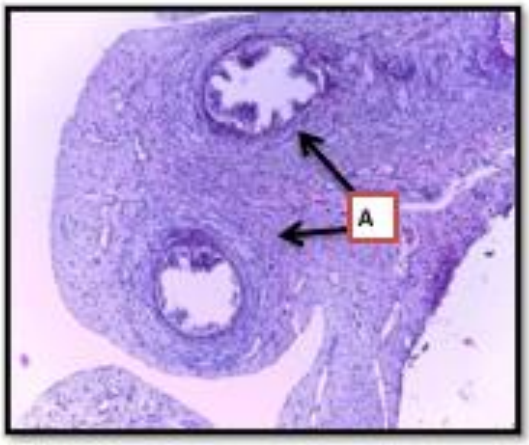

Figure (5): Cross section of the ovary in the (Clomid 2 months group) showing increasing in thickness of the germinal layer around the graafian follicule (A) (H&E) (100X).

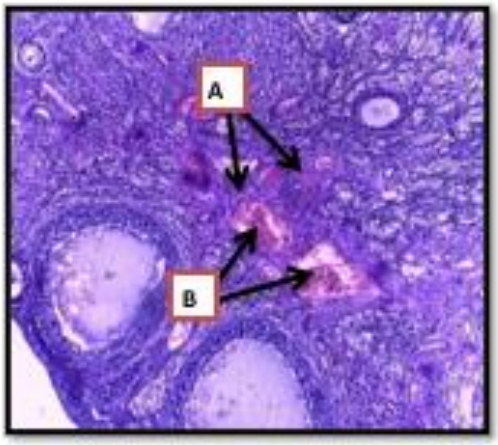

Figure (6): Cross section of ovary in (Clomid 2 months group) showing edema (A) congestion (B) (H&E) (100X).

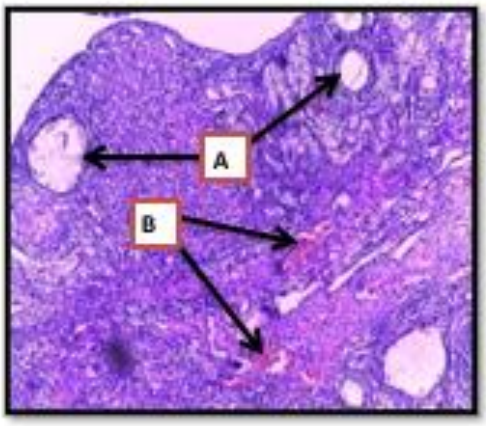

Figure (7): Cross section of the ovary in the (Duphaston 1 month group) showin a decrease in the number of follicles (A) congestion (B) (H&E) (100X).

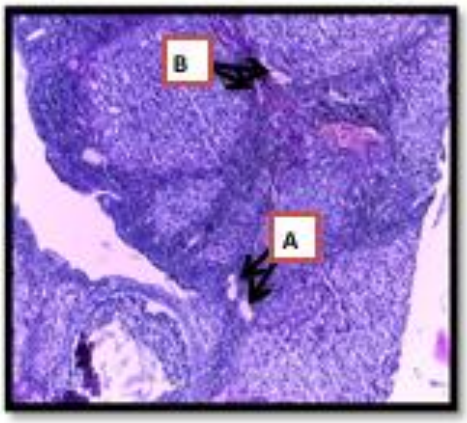

Figure (8): Cross section of the ovary in the (Duphaston 1 month group) showing vaculation (A) bleeding (B) (H&E) (100X).

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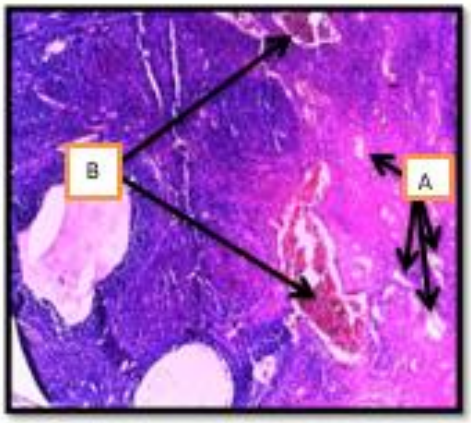
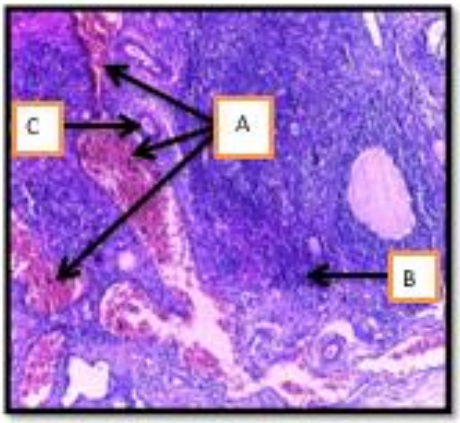
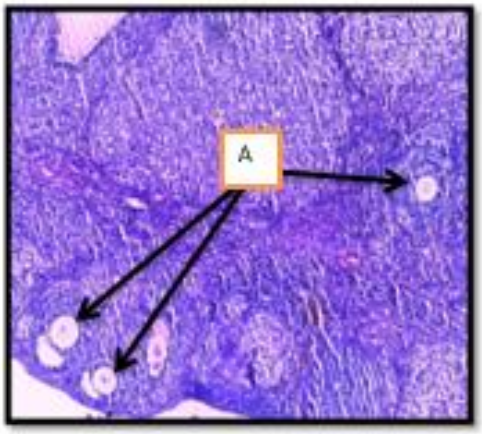


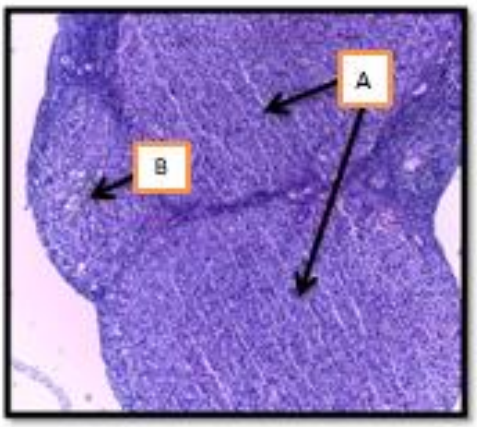
Figure(9):Cross section of the ovary in the (Duphaston 2 months group) showing necrosis (A) congestion (B)(H&E)(100X)



Figure(10):Cross section of the ovary in the (Duphaston 2 months group) showing severe conhestion (A) infiltration(B) fibrosis (C) (H&E)(100X).



Figure(11):Cross section of the ovary in the (Procreation v 1 months group) showing normal tissue with follicles (A)(H&E)(100X



Figure(12):Cross section of the ovary in the (Procreation v 2 months group) showing normal tissue (A) and simple hemorrhage (B) (H&E)(100X).

Discussion

The current study reveated to in creasing in sex hormones (estrogen,progesteron and prolactin) in all groups that treated with fertility drugs as clomid ,duphaston and procreation V.This raising of sex hormones may be linked to fertility drugs exposure ,this is consistent with study of Abdelhafiz and Muhamad, [9] who indicated to clomid administration led to improvement of LH and FSH which causes synthesize and secretion of sex hormones as esterogen and progesterone.Also, the study of Schoolcraft et al. [10]

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confirmed to LH lead to ovulation stimulation and corpus luteum formation which reflected on progesteron secretion.The increase in blood prolactin levels associated with the use of fertility-stimulating drugs may be due to physiological reasons or thyroid dysfunction, also showed hyperprolactinemia can result from hypothyroidism in patients with polycystic ovary syndrome.

Histological examination of ovary section in fertility stimulants drugs groups causes histopthological lesions such as necrosis,fibrosis,bleeding,congestion,edema and decline of ovarian follicles numbers,as well as increasing in thickness of germinal layer and in flammation.These may be associated to fertility drugs intake as clomid,duphaston and procreation . This is similar to study of Chaube et al. [11] who mentioned to reduction of follicles in immature female rats were injected with clomid.Also, this is agree with stud of Duran and Raja, [12]who reported to normal levels of LH and FSH in non-treated with fertility stimulants caused stimulation of follicle growth and ovulation.The increase in ovarian follicle size may be due to the proliferation of granulosa cells,leading to enhanced follicular growth and increased estrogen production [13].This aligns with thefindings of Michail et al. [14],who reported that clomiphene citrate increases the risk of granulosa cells tumors, especially when used in high doses.Continuous ovulation resulting from the use of fertility drugs may increase risks by preventing the ovarian surface epithelium from having non-ovulatory rest periods. The epithelial disruption created at the site of ovulation may serve as a source of cancerous cells.This aligns with study of Ozdemir et al. [15], who reported two previous cases of ovarian cancer following ovulation induction.Additionally, the Carter study [16] explained the causes of cancer as a result of trauma to the ovarian surface due to continuous ovulation.

Conclusion

The frequent use of ovulation-stimulating drugs can have a significant impact on various sex hormones in the body, including estrogen, progesterone, and prolactin, in addition to their harmful effects on ovarian tissue. It has been found that long-term use of these medications has a more negative impact compared to short-term use. Many studies have investigated the effects of these drugs on women undergoing fertility treatments and have highlighted their potential consequences. It has been observed that

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the use of ovulation-stimulating drugs can alter the levels of sex hormones in the body due to the presence of synthetic hormones similar to those naturally produced by the body.

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