

HISTOPATHOLOGICAL STUDY OF R- FSH EFFECTS ON FEMALE REPRODUCTIVE SYSTEM, LIVER AND KIDNEYS IN RATTUS NORVEGICUS RATS

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ABSTRACT

The present study aims to evaluate the histopathological changes induced by recombinant follicle stimulating hormone (*folitropin alpha*) on reproductive organs as well as the liver and kidneys of female rats. The experiment was done on 24 white female rats (*Rattus norvegicus*) sexually mature weighing 150-200 gram, divided into 4 equal groups of 6 animals: control group which was given distilled water. Single dose group, double dose group and triple dose groups which were injected by 0.5 iu., 1 iu. and 1.5 iu of recombinant FSH respectively. The drug was given subcutaneously during the pro-estrous phase for ten consecutive cycles, then animals from each group were sacrificed to study the histopathological changes. The histopathological examination of the ovaries, uterus, liver, and kidneys revealed variable changes in different organs. The ovarian sections showed many Graafian follicles without ova and many corpus luteal cysts, fibrosis, and thickened granulosa cell layer, and the ovary was surrounded by excessive adipose tissue. The uterus in single and double doses showed dilated cavity, thin endometrium, thin muscular layer and diminished endometrial glands while in triple dose showed atrophy of endometrial lining and glands, hypertrophied muscular layer with slit like endometrial cavity and formation of multiple endometrial cyst. The liver sections showed few changes like dilated central vein, congestion of sinusoids, vacuolation of hepatocytes, with moderate degree of fatty degeneration. A few hepatocytes appeared necrotic but without inflammatory response. The kidneys in single and double doses showed unremarkable changes, while in triple dose glomerular congestion, congested vessels, hemorrhage, and degeneration and necrosis of proximal tubules were found.

INTRODUCTION

The World Health Organization (WHO) estimates that 60 to 80 million couples worldwide currently suffer from infertility (1). Infertility is a common clinical problem. It affects 13% to 15% of couples worldwide, it is generally reported that in approximately 35% of cases, infertility is mainly due to a female factor (2). Infertility may be caused by an underlying medical condition that may interfere with ovulation, or causes hormonal complications. The therapy of infertility due to ovulation dysfunction aims to restore ovulation as physiological as possible. This involves the use of drugs and therapeutic protocols to obtain unifollicular cycles (3). This study deals with histopathological effects of recombinant follicle stimulating hormone (rFSH) of alpha type (*follitropin alpha*) on reproductive organs of rats after use in different doses. FSH is a glycoprotein hormone produced and stored in basophilic gonadotropin-producing cells (gonadotrophs) in the anterior pituitary together with LH, and is secreted by stimulation by a hypothalamic GnRH/LHRH (4).

In the female FSH is necessary for the selection and growth of ovarian follicles and for the production of estrogens from androgen substrates. FSH also stimulates maturation of the pre-antral follicle into the pre-ovulatory follicle by initiating granulosa cell aromatase activity and enzymes involved in progesterone biosynthesis. FSH also induces receptors for luteinizing hormone (LH) on granulosa cells, thereby allowing ovulation and development of the corpus luteum in response to the mid-cycle LH surge. FSH stimulation plays a critical role in follicular recruitment and dominant follicle selection (5,6).

MATERIALS AND METHODS

Determination of estrous cycle phases by vaginal lavage method

This method consists of flushing cells from the vaginal lining by introducing a small amount of normal saline into the vagina using a pipette and placing one or two drops of the resulting cell suspension onto a slide. The slides can be stained and then read (7).

Experimental animals

The experiment was conducted in the laboratory animals' house of Veterinary Medicine College - University of Basra. Where 24 white rats (*Rattus norvegicus*) sexually mature and 150-

200 gram weighting, were used. The animals were accommodated in the same laboratory condition by keeping them in special cages. The room temperature was set between 20-25 °C. The nutrient for rats was pellet and watered libitum.

In this experiment 24 *rattus norvegicus* female rats were divided into 4 equal groups of 6 animals for each: control group, single dose group, double dose group and triple dose groups.

All Animals were given medication as follows:

Group 1 (control group) were given drinking distilled water.

Group 2 (single dose group) were injected with 0.5 iu of the drug subcutaneously which equivalent to 150 iu human dose.

Group 3 (double dose group) were injected 1 iu of the drug subcutaneously which equivalent to 300 iu human dose.

Group 4 (triple dose group) were injected 1.5 iu of the drug subcutaneously which equivalent to 450 iu human dose.

(The dose calculated depending on the body weight of rats considering adult human weight 70 kg).

RESULTS

Gross examination

Gross examination of internal organs revealed progressive enlargement of the liver, spleen, and kidneys associated with pallor in treated animals compared to control animals. Also excessive amount of adipose tissue surrounding the reproductive organs was noticed, whereas, in one of the animals treated with triple dose a large bloody cyst measuring 2x2 cm. with edema of the reproductive system was found.

Histopathological examination

The histopathological study of the ovaries, uterus, liver, and kidneys revealed many changes caused by the drug on these organs. The ovarian sections showed many Graafian follicles without ova and many corpus luteal cysts, fibrosis, and thickened granulosa cell layer, while the ovary was surrounded by excessive adipose tissue as shown in figures (1,2,3&4).

The uterus in single and double doses showed dilated cavity, thin endometrium, thin muscularis and diminished endometrial glands while in triple dose showed atrophy of endometrial lining and glands, hypertrophied muscular layer with slit like endometrial cavity and formation of multiple endometrial cyst as shown in figures (5,6,7&8). The liver sections showed

many changes like dilated central vein , congestion of sinusoids , vacuolation of hepatocytes , with moderate degree of fatty degeneration. A few hepatocytes appeared necrotic but without inflammatory response as shown in figures (9,10,11&12). The kidneys in single and double doses showed unremarkable changes, while in triple dose glomerular congestion, congested vessels, hemorrhage, and degeneration and necrosis of proximal tubules were found as shown in figures (13,14,15&16).

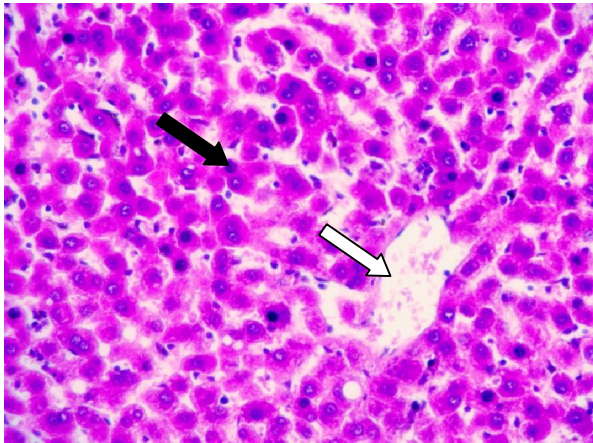


Fig. (1) Liver section in control group shows normal central vein(white arrow), normal hepatocytes (black arrow). (H&E) 400X.

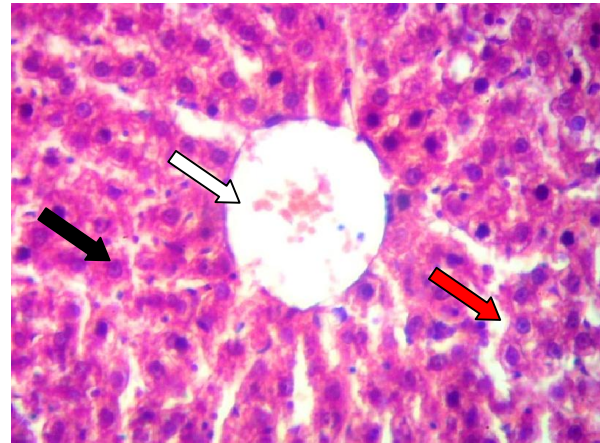


Fig. (2) Liver section in **single** dose group shows dilated central vein(white arrow) ,normal hepatocytes (black arrow) with dilated sinusoids(red arrow), mild to moderate congestion .(H&E) 400X.

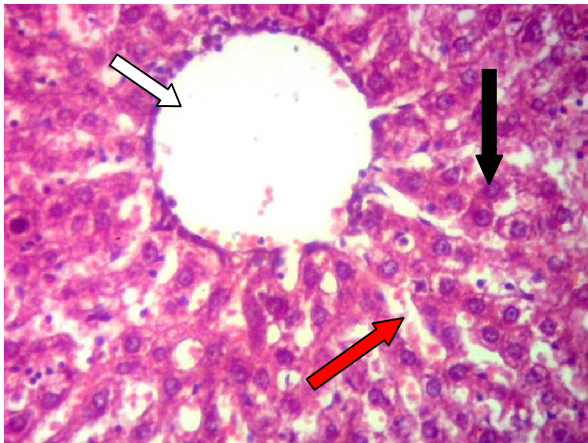


Fig. (3) liver section in double dose group showing droplet-like vacuoles within the hepatocytes (black arrow), congested sinusoids (red arrow) with dilated central vein(white arrow) .(H&E) 400X.

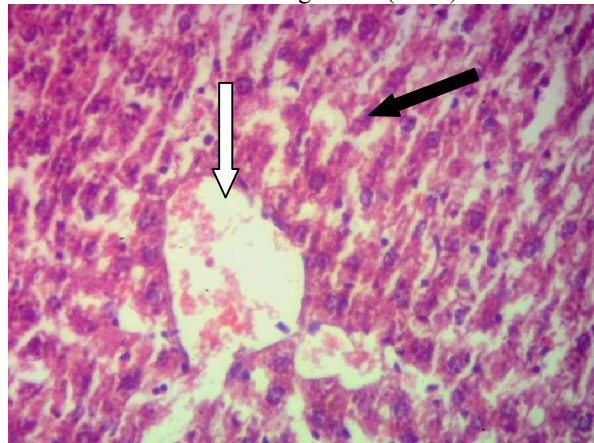


Fig. (4) Liver section in triple dose group Dilated central vein (white arrow) with moderate degree of fat degeneration (black arrow), (H&E) 400X.

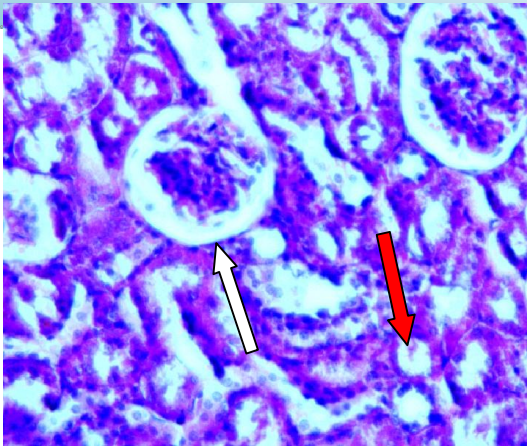


Fig. (5) kidney section in control group shows normal glomeruli (white arrow), and tubules (red arrow). (H&E) 400X.

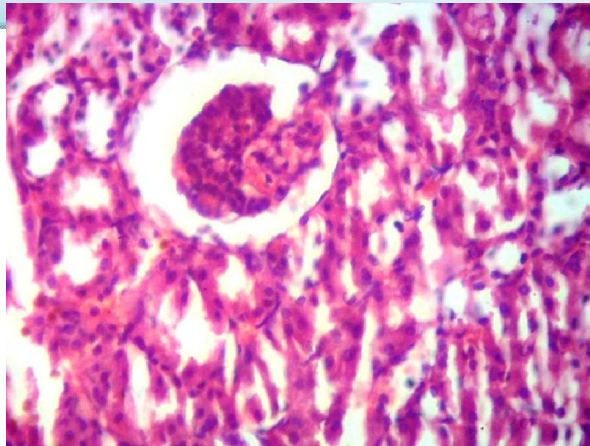


Fig. (6) Kidney section in single dose group shows unremarkable changes. (H&E) 400X.

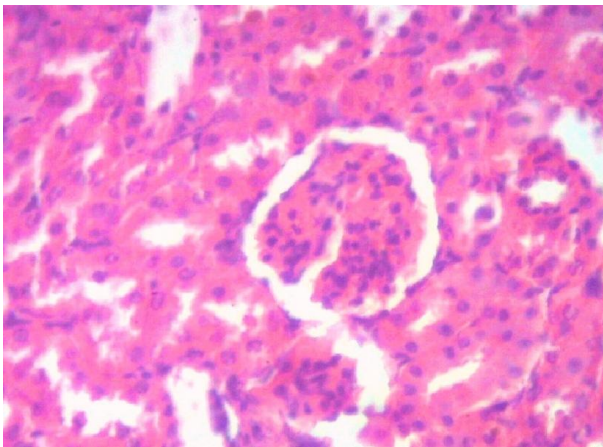


Fig. (7) Kidney section in double dose group showing congested vessels, normal renal tubules, glomeruli. (H&E) 400X.

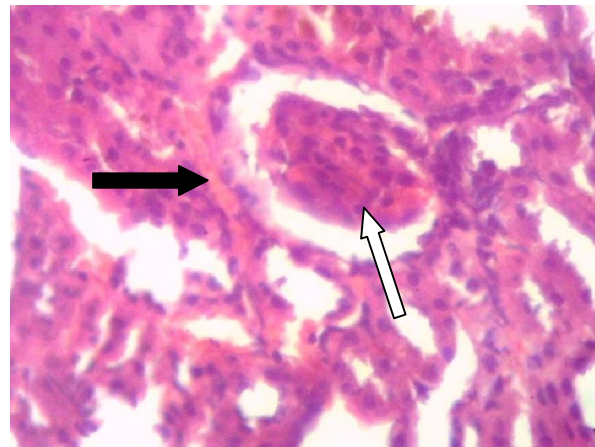


Fig. (8) Kidney section in triple dose group showing glomerular congestion (white arrow), congested vessels (black arrow), hemorrhage, degeneration and necrosis of proximal tubules. (H&E) 400X.

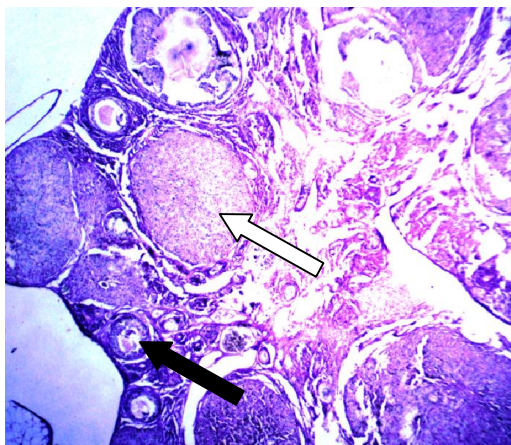


Fig. (9) ovary section in control group shows normal ovarian tissue, normal follicles (white arrow) and corpus luteum (black arrow). (H&E) 100X.

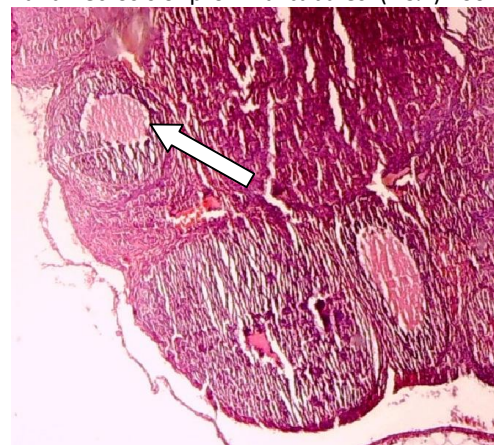


Fig. (10) Ovary section in single dose group shows many Graafian follicles without ova (white arrow) and many corpus luteum. (H&E) 100X.

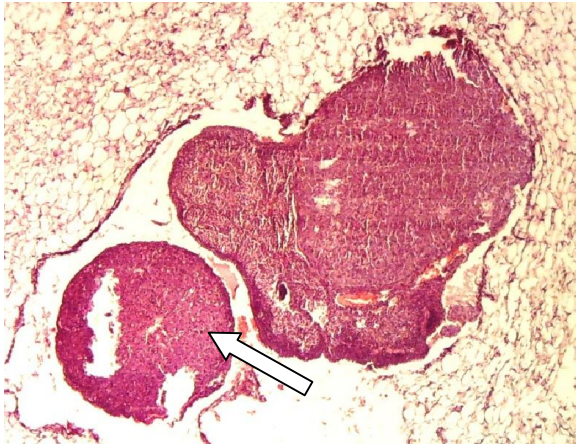


Fig. (11) Ovary section in double dose group shows absence of graffian follicles, only corpus luteum cyst formed (white arrow). Stain (H&E) 40X.

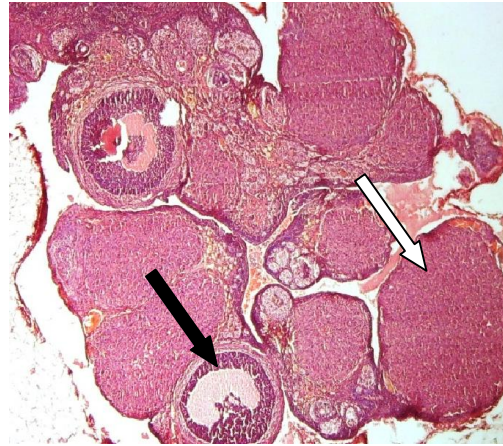


Fig. (12) Ovary section in triple dose group shows many primordial follicles fully mature but without ova (black arrow), many large corpora lutea (white arrow) and cysts. stain (H&E)100X.

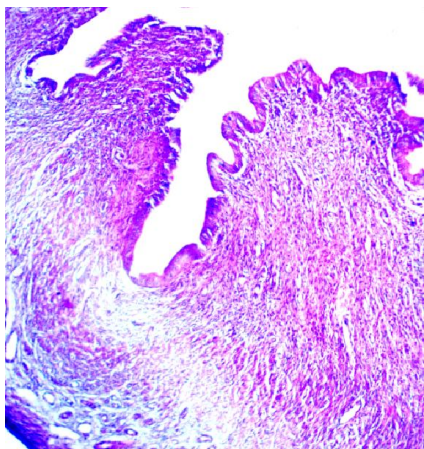


Fig. (13) Uterus section in control group. Stain (H&E) 400x.

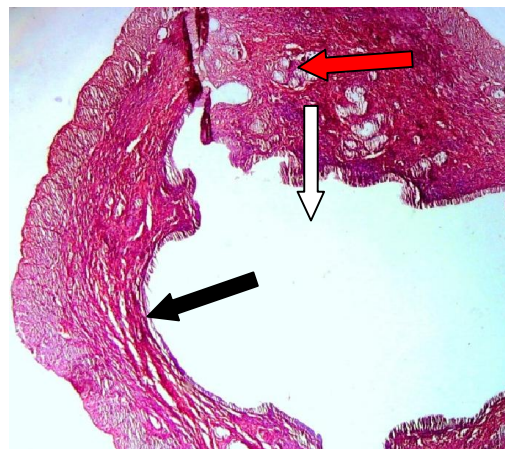


Fig. (14) Uterus section in single dose group shows normal lining (black arrow) and dilated lumen (white arrow) with normal glands (red arrow). Stain (H&E) 400X.

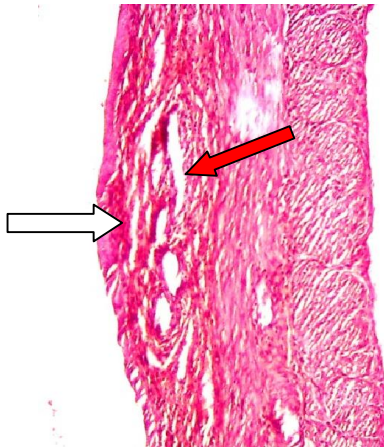


Fig. (15) Uterus section in double dose group shows, thin endometrium (white arrow) , thin muscularis and diminished endometrial glands (red arrow). Stain (H&E) 100X.

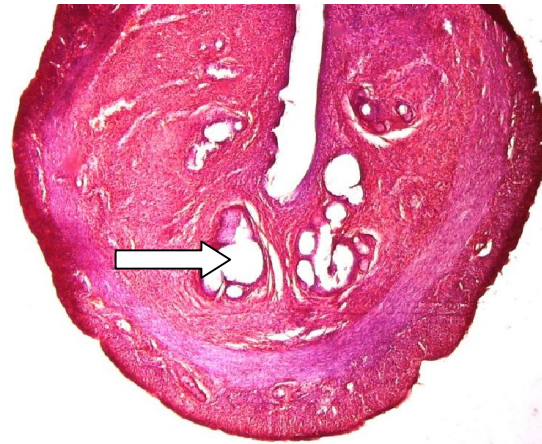


Fig. (16) Uterus section in triple dose group shows atrophy of endometrial lining and glands hypertrophied muscular cystic endometrium (white arrow). stain (H&E) 40X.

DISCUSSION

Ovulation failure is the primary defect in at least 20% of infertile couples. It is manifested as irregular /or infrequent ovulation or chronic anovulation as a result of deficient gonadotropins and their inability to stimulate follicle maturation.

Induction of ovulation by hyper-stimulation of the ovaries results in the selection of multiple oocytes in one cycle. The ova can be retrieved and used for *in vitro* fertilization. Ovulation induction therapy is directed at correcting the primary dysfunction causing anovulation by producing augmentation of endogenous gonadotropin (8).

Gross examination

Some studies report that fluid retention is a major side effect of the drug which may be the cause of enlargement of the internal organs like liver, spleen, and kidneys associated with pallor in treated animals compared to control animals may be due to edematous effects of the drug (9).

Histopathologic examination

In the present study the histopathological changes of the ovaries showed many graafian follicles without ova and many corpus luteal cysts, fibrosis, and thickened granulosa cell layer, while the ovary was surrounded by excessive adipose tissue. It was pointed that treating rats with

clomid (ovulation induction drug) at doses 10, 50 and 100mg for a week induced degenerative effects in the ovary, the ovarian stroma contained large number of vacuoles, atretic follicles of

different sizes and congested blood vessels, abnormal Graafian follicles appeared with enlarged antrum and degenerated zona pellucid. Morphometrical results indicated significant decrease in the number of ovarian follicles and increase in atretic ones (10). Another study showed that clomid caused ovarian and uterine abnormalities (11). It also induce apoptosis and degeneration in fallopian tube (12).

The uterus in the low and medium doses showed dilated cavity, thin endometrium, and thin muscularis and diminished endometrial glands while in high dose showed atrophy of endometrial lining and glands, hypertrophied muscular layer with slit like endometrial cavity and formation of multiple endometrial cyst. It was stated that Ovulation induction therapy accelerates the maturation of the endometrial stroma and is often associated with a discrepancy between early secretory glands and an edematous or decidualized stroma with spiral arterioles (13).

The liver sections showed many non-specific changes like dilated central vein , congestion of sinusoids , vacuolation of hepatocytes , with moderate degree of fatty degeneration. A few hepatocytes appeared necrotic but without inflammatory response. The kidneys in the low and medium doses showed unremarkable changes, while in high doses, glomerular congestion, congested vessels, hemorrhage, and degeneration and necrosis of proximal tubules were found.

دراسة مرضية نسيجية حول تأثير الهرمون المحفز للحويصلات على الجهاز التناسلي والكبد والكلية لانات الجرذان

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الخلاصة

الهدف من هذه الدراسة هو لتقييم التغيرات النسيجية المرضية الناجمة عن الهرمون المحفز للحويصلات على الاعضاء التناسليه (الرحم والمبايض) بالاضافه الى بعض الاعضاء الداخليه (الكبد والكلية). انجزت هذه الدراسة على ٢٤ جرد من اناث الجرذان المختبريه ناضجه جنسيا تتراوح اوزانها بين ١٥٠ - ٢٠٠ غرام قسمت الى اربع مجاميع متساويه في كل مجموعه عدد ستة جردان كما يلي : مجموعة السيطره، مجموعة الجرعه المفرده، مجموعة الجرعه الثنائيه، مجموعة الجرعه الثلاثيه.

المجموعه الاولى (مجموعه السيطره) : تم تجريعها ماء مقطر فقط. المجموعه الثانيه (الجرعه المفرده)، المجموعه الثالثه(الجرعه الثنائيه)، المجموعه الرابعه (الجرعه الثلاثيه) : تم حقنهم ٥,٠ - ١ - ١,٥ وحده دوليه على التوالي. كان الحقن يتم تحت الجلد كل ٤ ايام في طور PRO-ESTROUS لمدة عشرة دورات متتاليه.

بعد انتهاء فترة التجريع تم التضحية بجرذان كل مجموعته لغرض دراسة التغيرات المرضيه النسيجييه. اظهرت دراسة التأثيرات المرضيه النسيجييه العديد من التغيرات على الرحم، المبايض، الكبد والكلى حيث اظهرت المقاطع المبيضييه العديد من جريبات غراف بدون بويضه والعديد من اكياس الجسم الاصفر، تليف و تثخن في خلايا الطبقة المحببه والمبيض محاطا بكميه كبيره من الانسجه الشحميه. الرحم في مجموعتي الجرعه المفرده والثنائيه يظهر تجويف متوسع، بطانه رقيقه، طبقه عضليه رقيقه ايضا و تناقص في الغدد الرحميه بينما في مجموعه الجرعه الثلاثيه يظهر ضمور في بطانه الرحم والغدد الرحميه مع وجود طبقه عضليه متضخمه. مقاطع الكبد تظهر العديد من التغيرات التي تشمل توسع في الوريد المركزي، احتقان في الجيوب الكبديه، تشكل فجوات في الخلايا مع وجود درجه متوسطه من الانحلال الدهني، القليل من الخلايا الكبديه تظهر متآكله بدون استجابته التهابيه. اما الكلئ في مجموعتي الجرعه المفرده والثنائيه تظهر تغيرات غير ملحوظه اما في مجموعه الجرعه الثلاثيه تظهر احتقان في الاوعيه الدمويه الكببييه، نزف، وانحلال وتآكل في النبيبات الكلويه القريبه.

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