Response Of Ovarian And Uterine Morphological-Functional Features To Gonadotropins In Dexamethasone Treated Adult Mice

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Abstract

This study aimed at investigating the possibility of reversing the adverse effects, on the reproductive system, that are associated with the administration of synthetic glucocorticoids. Cycling Swiss albino mice were divided into four groups, 12 mice each. Control mice were daily intramuscularly injected with 0.1 ml normal physiological saline solution for ten days. Mice of the Gonadotropins group received six daily injections of 0.1 ml normal saline solution followed by three daily injections of the gonadotropins: 04 IU FSH activity on the seventh and ninth days, whereas 05 IU LH activity was administered on the tenth day. Mice of the Dexamethasone group were daily injected with 02 ug of the synthetic glucocorticoid for six days, followed by daily injections of 0.1 ml normal saline solution for four days. Animals of the Dexamethasone – Gonadotropins group were treated with Dexamethasone for the first six days followed by three daily injections of the Gonadotropins as described above. On the eleventh day, animals were euthanized with ether and weighed. Ovaries and uteri were dissected out, weighed and out into a tissue fixative solution to prepare stained sections.

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Administration of the gnadotropins resulted in a significant increase in the relative combined ovarian-uterine weight (mg/ 100 g body weight). On the other hand, Dexamethasone treated mice experienced significant decrease in the relative weight as compared with the corresponding weight in the control and gonadotropins groups. Injections of the gonadotropins following dexamethasone injections, improved the relative combined ovarian-uterine weight, but the extent of improvement failed to reach that of the gonadotropins group. The relative combined ovarian-uterine weight of the Dexamethasone-Gonadotropins group was significantly less than that of the control group and significantly higher than that of the Dexamethasone group ($P < 0.05$). Therefore, as far as the relative combined ovarian-uterine weight is concerned, gonadotropins could help in recovery from the adverse side effects of treatment with therapeutic daily doses of the synthetic glucocorticoid for a duration of six days, a period that well covered that of an estrous cycle.

Microscopic examination of the stained sections of the ovaries and uteri came out with indications that the structural-functional parameters were in favor of retained capability to positively respond to goadotropins following the suppressive effects of dexamethasone. Dexamethasone inhibited follicular development and luteinization. Subsequent administration of the gonadotropins improved the structural-functional picture of the ovaries. Nevertheless, the extent of this response to gondotropins was not parallel to that observed in mice into which gonadotropins alone were administered. Almost similar patterns of findings were observed in the uterine sections. Dexamethasone impaired morphological-functional development of the uterine endometrial stroma, uterine glands and endometrial epithelium. Gonsdotropins injections following dexamethasone treatment were capable of reversing the

suppressive effects of the synthetic glucocorticoid to a considerable extent. However, the response of the dexamethasone treated uteri to gonadotropins could not reach the response of the normal saline solution treated uteri to gonadotropins. The results were discussed on the bases of the possible indirect and direct effects of the glucocrticoids on the ovary and uterus. Impairment of the biosynthesis of ovarian steroid hormones could further participate in the induction of the reported uterine morphological functional events.

Key words: Glucocorticoids; Dexamethasone; Follicle Stimulating Hormone; Luteinizing Hormone; Ovary; Uterus.

Introduction

Early in the thirties of the last century, Edward Kendall in the laboratories of the Mayo Foundation, USA, isolated six hormones from the beef adrenal gland. He gave them the names: compound A, B, C, D, E and F. In 1935, the word "cortisone" was used to describe "Kendall's compound E" and referred to as 17-hydroxy-11-dehydrocorticosterone. In September, 1948, cortisone, also known as compound X, was intramuscularly injected for the first time into a rheumatoid arthritis patient. Nobel Prize was awarded, in 1950, to Kendall and other workers who participated in presentation of cortisone, the first identified and medically employed glucocorticosteroid hormone of the adrenal cortex (Ingle, 1950; http://www.myoclinic.org/tradition-heritage-artifacts/67-1.html, the official web site of the Mayo Foundation, accessed January 6, 2012).

Then after, other glucocorticoids were isolated and identified. Glucocorticoids, presently are considered as a class of steroid hormones whose most potent natural representative is cortisol (hydrocortisone).

They bind to their own receptors. The receptors are present in almost every vertebrate animal cell. They could be synthesized from cholesterol by P450-hydroxylases (Binkley, 1995). Glucocorticoids that are synthesized and secreted from the adrenal cortex, act at the cellular levels of the effecter organs including hypothalamus, pituitary gland and male and female reproductive organs. They have physiologic effects on metabolism, inflammation and immunity (Turner and Bangara, 1976; Binkley, 1995; Costanzo, 1998; Barrett *et al.*, 2010; Hall, 2011).

Beside the isolated naturally occurring glucocorticoids, synthetic steroids have also been marketed by the pharmaceuticals. Synthetic Glucocorticoids are widely used as part of treatment formulas for several abnormalities. Nevertheless, as any other drugs, administration of glucocorticoids has side effects that are represented by unwanted changes in morphological, biochemical and functional parameters of one or more organs of the body systems. Prednisolone has been used to reduce serum and follicular fluid androgenic hormone concentration in women suffering from Polycystic Ovary Syndrome (Fridstrom *et. al.,* 1999). Cortisol negatively influenced function of the oviduct (Andersen, 2002). Prednisolone reduced the number of uterine natural killer (uNk) cells in the endometrium (Tang *et. al*., 2009). Large doses of Dexamethasone, another widely employed synthetic glucocorticoid, had also induced changes similar to those induced by large doses of prednisolone (Birnbaum, 2008). Whirledge and Cidlowski (2010) reported that elevation of glucocorticoids levels in mammalian species could inhibit reproduction. Glucocorticoids actions could take place at multiple levels in hypothalamo – pituitary – gonadal axis. As a result the following changes might be resulted: 1. decreased synthesis and release of the hypothalamic gonadotropin releasing hormone (GnRH), 2. reduced

release of the pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH), 3. negative modulation of the testicular and ovarian steroidogenesis and/or gametogenesis, indirectly and directly.

Increased endogenous glucocorticoids, due to stress, in male rodents (rats and mice) was associated with lowered levels of prolactin, FSH and LH (Lopez-Calderon *et al*., 1984; 1987; 1989). In sheep, elevation of plasma cortisol, as in stress, caused suppressed GnRH secretion, reduced pituitary response to GnRH, reduced LH pulse frequency and impaired ovarian cyclicity (Oakley *et al*., 2009).

Disruption of the adult female rats' ovarian function could be induced by a single dose of dexamethasone. Disruption covered ovarian steroid hormones' levels, ovarian morphology and availability of hormonal receptors (Illera *et al*., 2005). Administration of dexamethasone in the rats resulted in reduced patterns of the ovarian estrogen and progestin secretion (Van Merris *et al*., 2007). In farm animals, Asa and Ginther (1982) investigated the side effects of glucocorticoids in the mare. Dexamethasone suppressed LH, follicular development, estrus and ovulation. Later on, Ferris and McCue (2010) reported decreased uterine edema, suppression of LH and a high incidence of ovulation failure in mares that were treated with dexamethasone. In dairy cows, treatment with betamethasone, another synthetic glucocorticoid, caused reduction in the life span of corpus luteum and decrease in response of the pituitary release of LH to the administered exogenous GnRH (Dobson *et al*. 1987).

The present trial aimed at: 1 – Identification of the possible changes in structural-functional parameters of the ovary and uterus, which could be associated with intramuscular administration of therapeutic dose of a synthetic glucocorticoid, Dexamathasone, for a

period of time that exceeds the duration of one estrous cycle. 2 – Exploration of the response of the Dexamathasone treated ovarian and uterine structural-functional parameters to exogenous gonadotropins (Follicle Stimulating Hormone, FSH, and Luteinizing Hormone, LH, activities) that are administered during a period covers the duration of one estrous cycle.

Materials and Methods

1. Animals: Fertile adult (around three months old) female mice were obtained from and treated at the Laboratory Animals' House of the Faculty of Medicine, University of Benghazi. They were accommodated, in hard plastic cages (Dimensions: 36 X 35 X 19 cm), four in each cage, in a room whose temperature was around 25 ± 2°C. The light periods were those of the day time. They were freely offered a locally available pellet diet supplied by the Animals Feed Plant, Benghazi. Animals had *ad libitum* access to water which was supplied in bottles with proper nozzles. Diet was checked for sufficiency and water was replaced daily. Cages were cleaned and their wooden fine flakes were replaced twice a week.

2. Chemicals: Chemicals, that were used to prepare the tissue fixative solution and the stains, were of laboratory grade. Drugs were purchased locally. Dexamethasone, 4 mg of the synthetic glucocrticoid (equal to 5 mg dexamethasone sodium phosphate) in 1.0 ml sodium phosphate solution, was supplied by Organon, Oss, Holland under the trade name Oradexon. The human menopausal gonadotropin (menotropin) was used for its follicle stimulating hormone (FSH) activity to stimulate follicular development. It was manufactured by Ferring Pharmaceuticals, Kiel, Germany. Each ampoule contained dried menotropin corresponding to 75 International Units (I.U.) of follicle stimulating hormone (FSH). The

preparation is marketed under the trade names Menopur and Menogon. van der Weijer *et al*. (2003) confirmed presence of contaminants including 1:1 luteinizing hormone (LH). LG Life Sciences, South Korea was the supplier of the vials containing 5000 I.U. of dried human chorionic gonadotropin (HCG), a placental polypeptide hormone whose action is identical to that of the pituitary luteinizing hormone (LH). The vials carried the trade name IVF-C.

3. Preparation of the diluted solutions of hormones: The hormones ampoules and vials were handled as if they were representing therapeutic doses for a human female weighing 60 kilograms (Dexamethasone: 04 - 08 mg; Menogon (FSH-LH): 75 - 300 I.U.; IVF-C (HCG): 5000 - 10000 I.U.). The initial body weight of the employed mice was not less than 25 g and up to about 31 g. The average mice weight was initially about 27 g. Doses of the injected hormones were calculated according to ranges of the therapeutic doses and according to the mean of the initial weight with slight corrections to facilitate dilution steps. Normal physiological saline solution was used to dilute the hormones preparations. Dilution was adjusted so that the calculated dose was presented in a volume of 0.1 ml.

4. Injection protocol: Vaginal smears were prepared to confirm phases of the estrous cycle. Forty eight normally cycling female mice were randomly selected, allocated to the following four groups, 12 mice each, and then the injection protocol started after an adaptation period of five days:

a. **Control group:** on the next day following the adaptation period, mice of this group were intramuscularly injected with 0.1 ml normal physiological saline solution for ten days.

b. **Gonadotropins group:** whose animals received intramuscular injections of 0.1 ml normal physiological saline solution for six days followed by intramuscular injection of the menogon, 04 I.U. in 0.1 ml, on the seventh and ninth days. Then, on the tenth day a single intramuscular injection of the HCG, 05 I.U. in 0.1 ml, was administered.

c. **Dexamethasone group:** animals of this group were intramuscularly injected, for six days, with 02 ug Dexamethasone in 0.1 ml, followed by injection of 0.1 ml normal saline solution on the seventh, ninth and tenth days.

d. **Dexamethasone- Gonadotropins group:** mice of this group were intramuscularly injected, for six days, with the calculated daily dose of dexamethasone. On the seventh and ninth days, they received the calculated daily dose of menogon followed by a single intramuscular injection of the HCG calculated dose on the tenth day.

5. Tissue sampling: During the whole injections period, animals were daily observed for changes in behavior. Twenty four hours after the last injection, animals of all groups were individually weighed and then euthanized with ether. Ovary-Uterus, as one piece, was dissected out, blotted with filter paper to remove fluid and then weighed. The combined ovarian-uterine weights were expressed as relative weights: combined ovarian-uterine weight in mg / 100 g body eight. Then, the dissected out structures were washed with normal physiological saline solution and transferred into formalin- acetic acid- alcohol fixative solution.

6. Preparation of stained histological sections: The fixed ovaries and uteri were separated from each other. Representatives of the fixed uteri of the four experiment groups were photographed. Whole ovaries and pieces with suitable size from the mid portions of the uteri were processed to prepare hematoxylin- eosin stained sections according to steps described by Humason (1981).

7- Microphotography: The light microscope, with suitable magnification powers, was employed to carefully examine the structural-functional features of the hematoxylin-eosin stained ovarian and uterine tissues sections. Selected representative sections were microphotographed. A light microscope-digital camera set up was available for microphotography. A personal computer main unit, with built-in TV-capture card, was connected to the digital camera. A micrometer stage slide with an engraved ruler of 2.0 mm long in 0.1 mm divisions was used to determine the magnification powers that were used in microphotography.

8. Statistical analysis: Numerical data of the ovarian and uterine relative weights were subjected to statistical analysis in order to test the significance of differences between means. The student t-test (Fowler and Cohen, 1997) was the test of choice to compare relative weights means of the mice groups. Means were tabulated as mean ± standard deviation (SD). The calculated P values were included within the table. Calculated P values of less than 0.05 were considered quite enough to indicate significance of the difference between compared means.

Results

Throughout the experiment period, the mice showed normal behavior, especially their apparent interest in food and water. All animals, for limited period of time, showed signs of unrest following the intramuscular injection of the specified solution. During this short period the animals moved around and tried to lick the site of injection. Sites of injections did not show pathological lesions. Changes in social attitudes of the animals toward each other, within each cage, were not noticed.

1. Relative Combined Ovarian-Uterine Weight: Means and their standard deviations of the body weight, absolute combined ovarian-uterine weight and relative combined ovarian-uterine weight of the control and treatment groups have been listed in Table 1. P values of the statistical analyses of the relative combined ovarian-uterine weight have also been included in Table 1. The tabulated results pointed out to a significant increase in mean of the relative combined ovarian-uterine weight following administration of the gonadotropins (355 ± 22.6 mg/100 g body weight) as compared with that of the control (307 ± 13.4 mg/100 g body weight). Significant reduction in mean of the relative combined ovarian uterine weight has been recorded in the dexamethasone treated mice (269 ± 13.1 mg/100 g body weight) in comparison with those of the control and gonadotropins treated animals. The relative combined ovarian-uterine weight of mice group which received six daily doses of dexamethasone followed by three daily doses of gonadotropins (330 ± 16.3) has shown a significant increased mean over than that of the dexamethasone group of mice. However, this mean was still significantly lower than that of the mice in both control and gonadotropins groups.

** → → Table 1.

Figure 1 shows selected representatives of uteri from the four groups of mice. The gross appearance of these uteri has reflected the effects of different treatments that have been employed. The gross morphology of the gonadotropins treated uterus has better developed appearance over than that of the control. The apparently less developed uterus was that of the dexamethasone treatment. Uterus of the dexamethasone – gonadotropins treated animal showed gross morphological improvement over than that of the control and dexamethasone alone, but still did not reach that of the gonadotropins alone.

** →→ Figure 1.

2. Ovarian Structural – Functional Parameters: Microscopic examination of the hematoxylin – eosin stained ovarian histological sections of the mice in the control group showed various stages of follicular development. The examined stained sections showed, also, corpora lutea of recent and previous ovulations as judged by the morphological appearance of the luteal tissue (Figure 2). Figure 3 represents a selected ovarian section of gonadotropins injected mice. Stained sections that were prepared from ovaries of the gonadotropins treated animals have been characterized with stimulated follicular development and formation of corpora lutea. Depressed follicular growth and luteinization have been the distinguished features of the ovarian stained sections of the mice in the dexamethasone treated group. The observed morphological – functional changes pointed out to impaired follicular development and consequent impairment of corpora lutea formation (Figure 4). Sections which were prepared from mice into which gonadotropins were administered after six injections of dexamethasone,

showed considerable activity in follicular development and formation of corpora lutea (Figure 5). The improvement in the morphological – functional parameters of these ovarian sections was positively distinguished as compared with the observed ovarian parameters of the normal saline (Figure 2) and dexamethasone (Figures 4) treated mice. At any rate, such characteristic feature could not parallel those of the corresponding features in ovarian sections of the gonadotropins treated mice (Figure 3).

** → → Figure 2.
Figure 3.
Figure 4.
Figure 5.

3. Uterine Structural – Functional Parameters: Morphological-functional features of the endometrial stroma, uterine glands and endometrial epithelium have been emphasized in the microscopic examination of the hematoxylin – eosin stained ovarian sections. Uterine sections of the control mice revealed expected morphological – functional parameters which reflected the normal response to hormones of cycling ovaries. Proliferating stroma, moderately developed uterine glands as well as endometrial epithelium were the predominant observed findings (Figure 6). Figures 7 and 8 represent stained sections in uteri of gonadotropins treated mice. Increased stromal profilation, uterine gland development and active columnar endometrial epithelium made their significant presence. The reversed picture was encountered in the uterine sections of the mice into which dexamethasone was administered for six days. Depressed proliferation of the stroma, absence of uterine glands development and inactive low cuboidal endometrial epithelium comprised the main features (Figure 9).

The outcome of the uterine sections microscopic observation pointed out to positive changes in the dexamethasone – gonadotropins group. Uterine sections exhibited increased proliferation activity in the stroma. The uterine glands and endometrial epithelium showed increased activity (Figure 10). The overall picture of the dexamethasone – gonadotropins uterine sections expressed noticeable improvement as compared with that of the sections prepared from control and dexamethasone treated mice. The extent of improvement in the components of these sections could not reach that of the gonadotropins mice.

** → → Figure 6.
Figure 7.
Figure 8.
Figure 9.
Figure 10.

Discussion

Results of the experiment pointed out to unrest signs and licking of the injection sites following the intramuscular administration of the treatment protocol as the only changes induced into the employed animals by treatment regimens. Administration of the gonadotropins as well as dexamethasone did not cause apparent changes in behavior including social activities of the four female mice of each cage. Furthermore, the results revealed lack of a noticeable impact for treatments on the animals' physical activity and their interest in the offered food and water. Aggressive behavior as well as maternal behaviors of the lactating rats were attenuated by dexamethasone (Vilela and Giusti-Pavia, 2011; Vilela *et al*., 2012). Betamethasone caused a significant reduction in locomotor activity of the adult rats (Pitzer and Schmidt, 2009). Depression-like behavior due to dexamethasone therapy has been reported in the offspring of the pregnant females when administration of the drug took place late in the gestation period (Hauser *et al*., 2009; Roque *et al*., 2011; Liu *et al*., 2012).

1. Relative Combined Ovarian-Uterine Weight: Changes in the relative combined ovarian-uterine weight, with absence of remarkable differences in means of the body weight, reflect fluctuations in the outcome of morphological, chemical and functional events. Events in the ovaries are ordinarily brought about by the gonadotropins, whereas those in the uteri are modulated by the ovarian hormones (Zarrow *et al*., 1964; Turner and Bangara, 1976; Costanzo, 1998). Therefore, as compared with the normal saline injected control mice, injection of two doses of FSH activity and one dose of LH activity would positively improve ovarian mass and function leading to similar response in the uteri. Consequently,

the relative combined ovarian-uterine showed the recorded significant increase. On the other hand, disruption, by dexamethasone, of the physiological release of the hypothalamic GnRH, release of the pituitary FSH and LH, and decline in ovarian steroidogenesis and oogenesis (Breen and Karsch, 2006; Whirledge and Cidlowski, 2010) would definitely cause lack of normal response of the uteri to ovarian hormones. In the mouse, dexamethasone inhibited the mitotic activities that are induced by estradiol and caused reduction in uterine weight (Gunin *et al*., 2001). The overall picture that might be induced by dexamethasone came along with the significant depression in the combined relative ovarian-uterine weight four days, a period close to the duration of the estrous cycle, after the last injection of the synthetic glucocorticoid. The approximate number of days at the end of which the hypothalamic-pituitary-ovarian-uterine axis might recover from the induced insult by administration of dexamethasone could be a point of future investigation. The results, in terms of the combined relative ovarian-uterine weight as well as gross morphology (Figure 1), might suggest significant recovery of the ovarian-uterine axis by exogenous gonadotropins administered during the same four days period following last dexamethasone injection.

2. Ovarian Structural – Functional Parameters: Administration of the normal saline could not interrupt the normal functional morphology of the ovaries in response to the fluctuating levels of the endogenous pituitary gonadotropins. The examined stained ovarian histological sections showed clear cut evidence of normal cyclic changes in structural-functional entities. Different developmental stages of follicles and corpora lutea (Figure 2) were substantial observed evidence. The expected remarkable improvement in ovarian morphological functional picture (Figure 3) was brought about by the administration of two doses

of the urine-isolated menopausal gonadotropin and the following single injection of HCG during four day period. Further activation of follicular development and luteinization after ovulation in response to exogenous FSH and LH has been documented and laid down in the basic textbooks of physiology and endocrinology (Turner and Bangara, 1976; Binkley, 1995; Hall, 2011).

Administration of six daily dexamethasone doses interrupted the cyclic histo-physiological events in the ovaries (Figure 4) on the fourth day following the last glucocorticoid injection. The remarkable inhibition in the ovarian functional morphology (Illera *et al*., 2005) could be due to both disruption of the GnRH and gonadotropins secretion (Whirledge and Cidlowski, 2010) and to suppression of ovulation mediators such as prostaglandins and plasminogen activator (Mikuni *et al*., 2009). The conclusions that were reached to by Ferris and McCue (2010), following their *in vivo* investigation in mares, emphasized dexamethasone – induced suppression of pituitary LH surge associated with a high rate of ovulation failure. Dexamethasone inhibited 3-beta-hydroxysteroid dehydrogenase, a key enzyme in progestins and estrogens biosynthesis of the porcine ovary (Jana *et al*., 2005). In the present study, introduction of exogenous FSH and LH activities during the four day period following last dexamethasone injection has achieved a considerable success in counteracting the suppressive influence of the synthetic glucocorticoid (Figure 5). These results have pointed out to the possibility of establishing ovarian recovery, following administration of therapeutic daily doses of dexamethasone for a period of six days, by means of exogenous FSH activity, two doses, and LH activity, one dose.

3. Uterine Morphological – Functional Parameters: The results revealed that the components of the uterine endometrial functionalis zone in the normal saline injected control mice showed normal appearance in response to estrogens and progestins of cycling ovary (Figure 6). The uterus, mainly the lamina propria, the uterine glands and lining epithelium of the endometrial functionalis zone are targeted by the ovarian hormones (Junquera and Carneiro, 2007; Paulson, 2011). The ovarian morphological – functional features in the ovary during the menstrual cycle of the primates and the estrous cycle of other mammals are associated with endometrial changes in response to ovarian hormones. Therefore, activation of the ovary by the exogenous gonadotropins had a positive impact on the uterine endometrium (Figures 7 and 8) represented by increased proliferation of the stroma and further development of the glandular and lining epithelium.

In the mouse, the structural – functional changes in the ovary and consequently the structural – functional changes in the uterine endometrium take place within the period of the estrous cycle; i. e. within a period of four days. Six daily injections of therapeutic doses of dexamethasone followed by four injections of normal saline were enough to disrupt the endometrial functionalis zone morphological – functional picture (Figure 9). These findings reflected the inhibitory action of dexamethasone on plasma estrogens (Konig *et al*., 2006), pituitary LH and ovarian estrogens and progestins secretion (Kalantaridou *et al*., 2004). Rae *et al*. (2009) referred to suppression effect of glucocorticoids on angiogenesis. In rats, the endometrial structural and immunological changes by estrogens were inhibited by dexamethasone (Rhen *et al*., 2003; Rhen and Cidlowski, 2006). Estradiol- induced increased mitotic activity in the mice endometrial glandular and lining epithelium was

counteracted by dexamethasone (Gunin *et al*., 2001). Disruption of the endometrial response to estrogenic and progestinic ovarian hormones could be due to the reduced patterns of these hormones secretion as reported in rats by Van Merris *et al*. (2007) following administration of dexamethasone. The observed changes in the mice endometrium of the present investigation were recorded on the fifth day after the last glucocorticoid injection. This period of time covered the four-day duration of the mouse estrous cycle. As this period was not enough for the ovaries to recover from the suppressive impact of the six dexamethasone injections, it is feasible to record such observed changes in the ovarian hormones- dependent uterine endometrium.

The administered exogenous hormones with FSH and LH activities have succeeded to a considerable extent in positively handling the suppressive actions of dexamethasone on the ovarian morphology and function. Therefore, it is reasonable to observe remarkable improvement in the endometrial parameters of the dexamethasone – gonadotropins treated mice. These observations pointed out to regaining of response of the endometrial components to the possibly reestablished secretion of the ovarian sex hormones (Figure 10). In other words, the results could be used to formulate a conclusion that the possible indirect and direct disruptive effects of dexamethasone on the uterine endometrium have been remarkably counteracted with the employed protocol of gonadotropins injections. The possibility of full recovery of both the ovaries and uterus endometrium after a second attempt of gonadotropins administration could not be overlooked.

Conclusion

Results of this investigation have confirmed unwanted effects of therapeutic doses of the widely used synthetic glucocorticoid, dexamethasone, for a period covers duration of more than one estrous cycle. The emphasized unwanted effects were gross morphology, relative weight as well as functional micromorphology of the ovary and uterus on the fifth day after the sixth injection of dexamethasone. Results showed the possibility of counteracting these recorded disruptive changes with a gonadotropins protocol including administration of two doses of FSH activity and one dose of LH activity during the four days following dexamethasone injections. This study may point out to the need for further studies that may involve concomitant estimation of the hypothalamic gonadotropins and ovarian sex hormones during days of the dexamethasone treatment, with different doses, and during the following days after cessation of dexamethasone therapy. Determination of the length of time that is required for the endogenous hormones to regain their normal values would be a good focus of study. Furthermore, the response of the pituitary hormones to exogenous GnRH in addition to changes in availability of receptors of the ovaries to gonadotropins and uterine receptors to ovarian hormones due to glucocorticoids therapy would also be interested areas of further studies.

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Table 1. Body weight (g), absolute combined ovarian – uterine weight (mg) and relative combined ovarian – uterine weight (mg / 100 g body weight) of the control, gonadotropins, dexamethasone and dexamethasone – gonadotropins groups of adult mice.

GROUPS	**Body Weight (g)**	**Absolute Combined Ovarian – Uterine Weight (mg)**	**Relative Combined Ovarian – Uterine Weight**	
			mg/100g	**P value**
Control C	27. 11 ± 1.20	83 ± 7.1	307 ± 13.4	
Gonadotropins T1	27.94 ± 1.27	99 ± 10.7	355 ± 22.6	C **X** T1: 0.00012
Dexamethasone T2	26. 91 ± 1.26	72 ± 3.5	269 ± 13.1	C **X** T2: 0.00023 T1 **X** T2: 0.00738
Dexamethasone–Gonadotropins T3	27. 10 ± 2.33	89 ± 12.1	330 ± 16.3	C **X** T3: 0.00038 T1 **X** T3: 0.01518 T2 **X** T3: 0.00027

Means ± SD. P value of less than 0.05 was considered as an indication of significant difference between the compared means. There were 12 adult cycling female mice in each group.

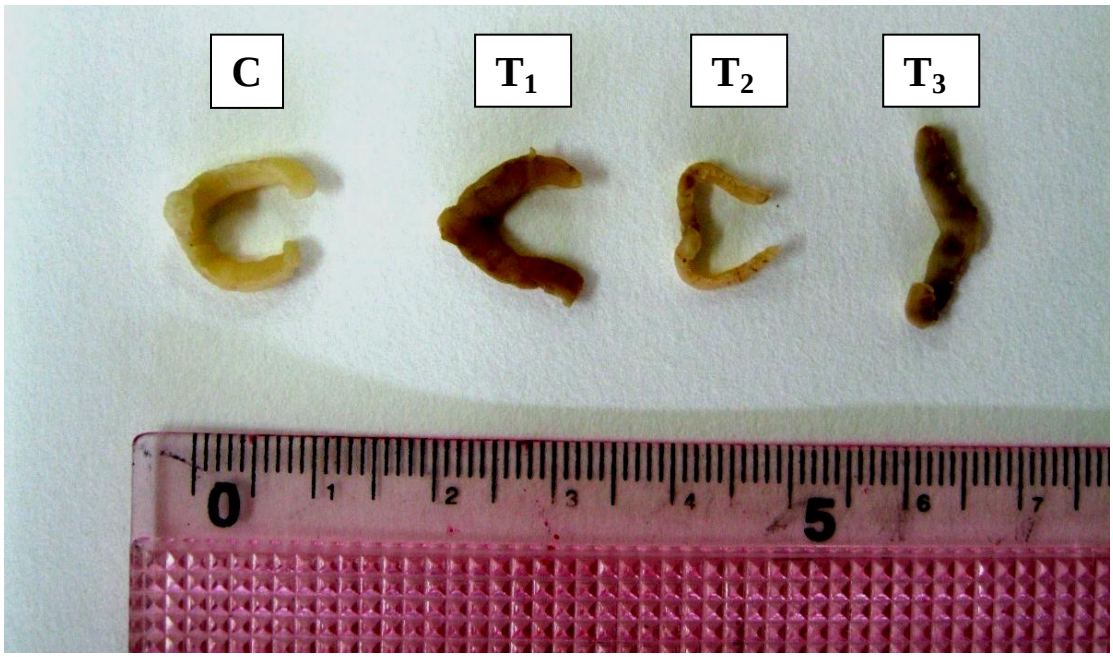


Figure 1. Selected uteri representing the control (C), gonadotropins (T1), dexamethasone (T2) and dexamethasone followed by gonadotropins (T3) groups of adult mice.



Figure 2. Hematoxylin-eosin (H-E) stained section in ovary of a control adult cycling mouse. Follicles (arrow head) with different stages of development as well as corpora lutea (arrow) are observed. (X 200).

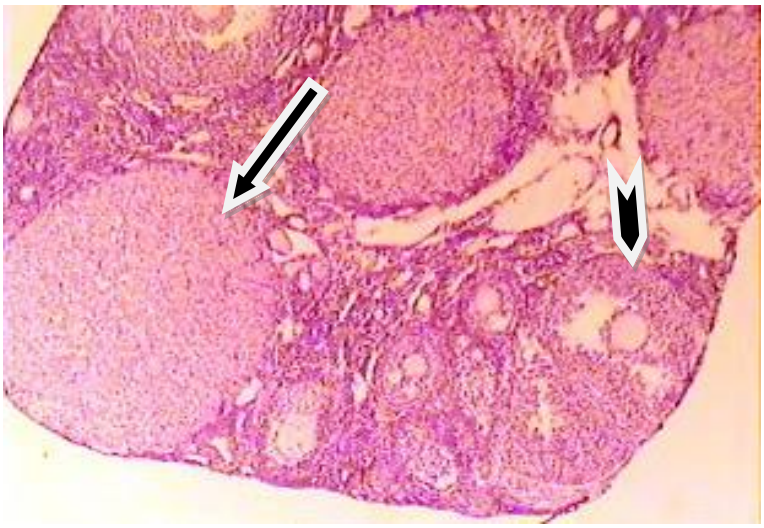


Figure 3. Ovarian stained section of a gonadotropins- treated adult mouse. Follicular development (arrow head) shows activity beside remarkable luteinization (arrow). (H – E, X 200).

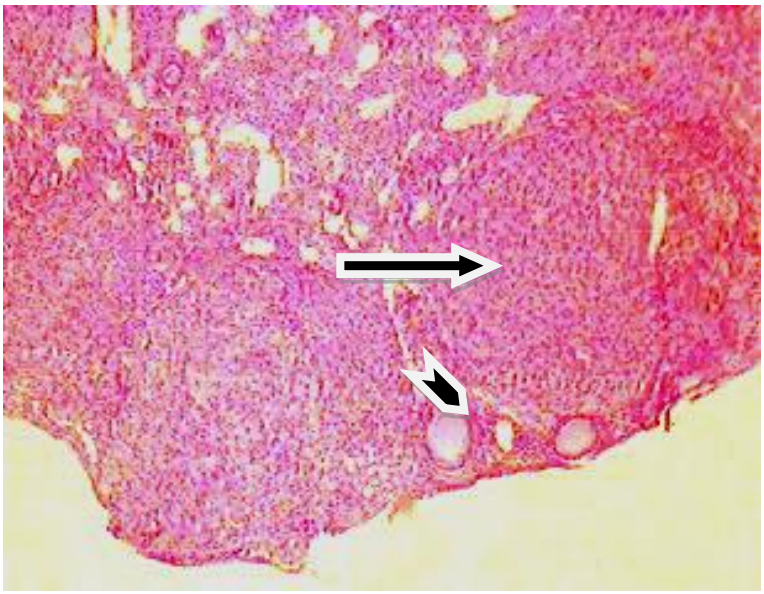


Figure 4. Stained section in ovary of a dexamethasone treated mouse. Follicular development (arrow head) and luteinization activity (arrow) are remarkably inhibited. (H – E, X 200).

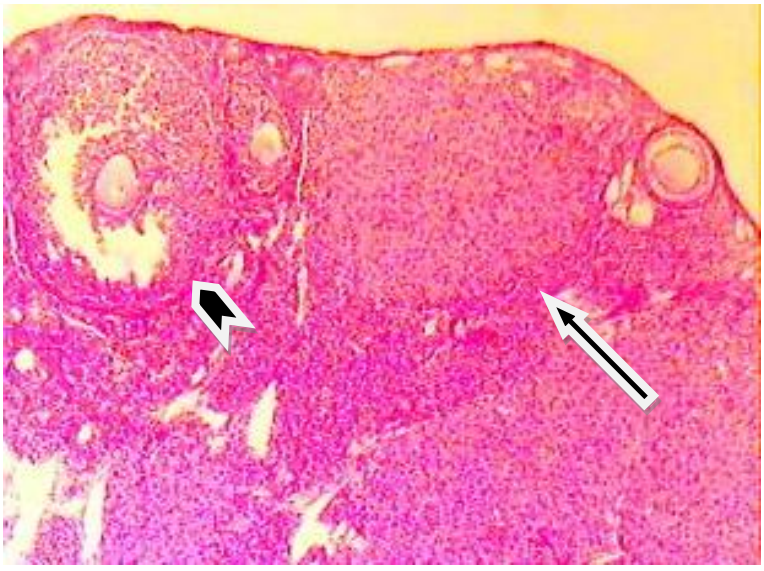


Figure 5. Moderate activity in follicular development (arrow head) following the injection of gonadotropins into dexamethasone treated mice. The ovarian section, also, shows presence of luteal tissues (arrow) with variable activities. (H – E, X 200).

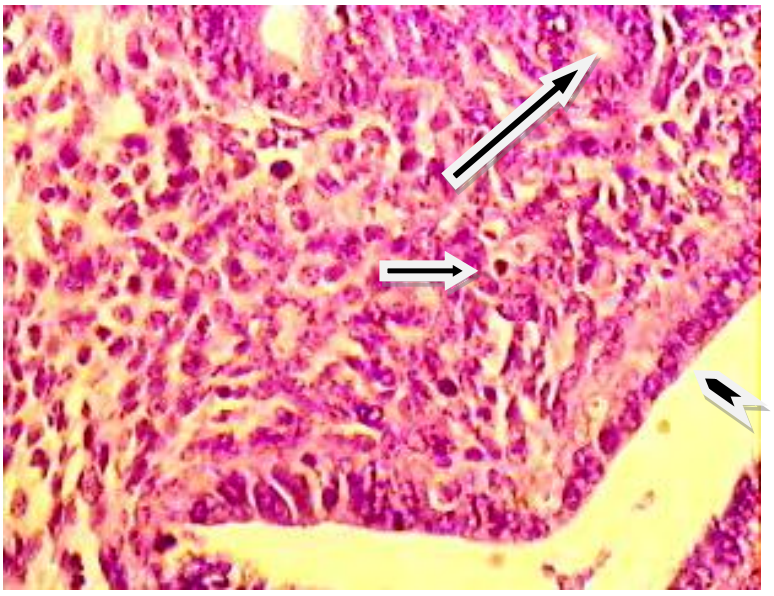


Figure 6. Stained histological section of a control mouse uterus. The endometrial epithelium (arrow head), stroma (short arrow) and uterine glands (long arrow) show the expected normal appearance of a cycling female. (H – E, X 400).

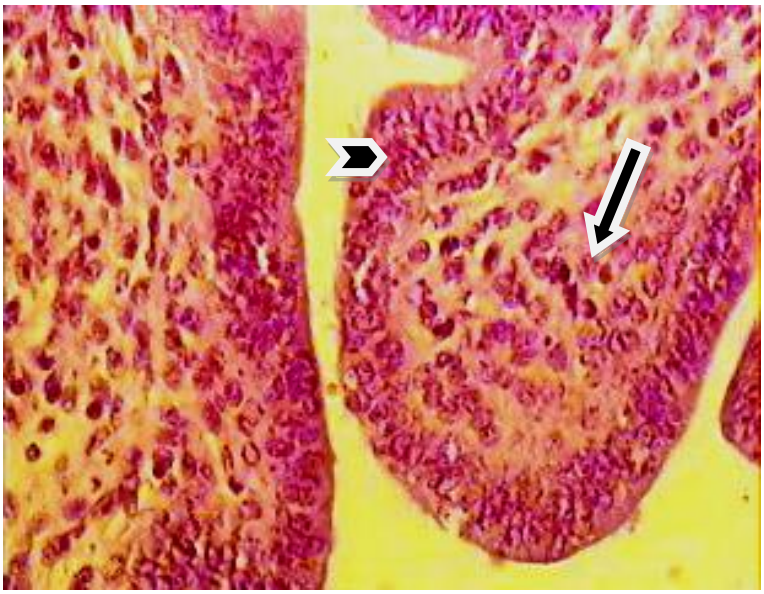


Figure 7. Improved endometrial epithelium (arrow head) and endometrial stroma (arrow) following injection of the gonadotropins into cycling adult mice. (H – E, X 400).

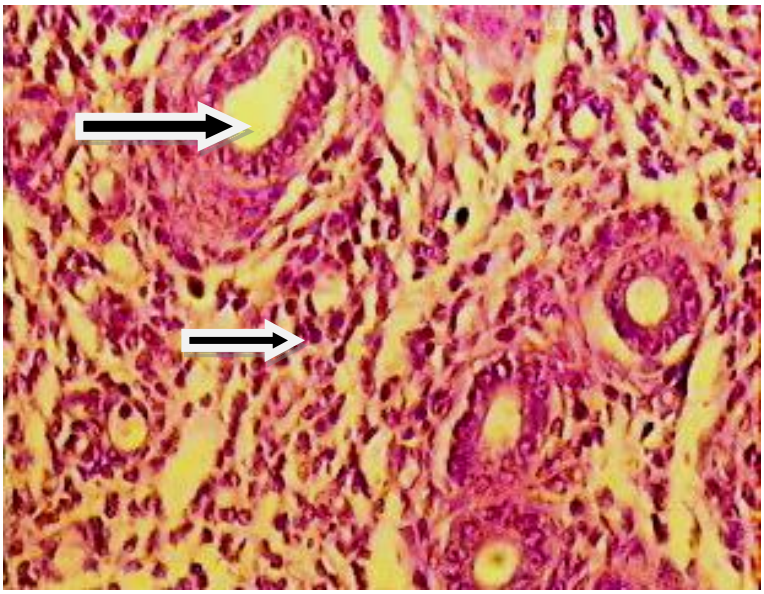


Figure 8. Proliferated endometrial stroma (short arrow) and increased development of the endometrial glands (long arrow) in uterine section of an adult mouse from the gonadotropins injected group. (H – E, X 400).

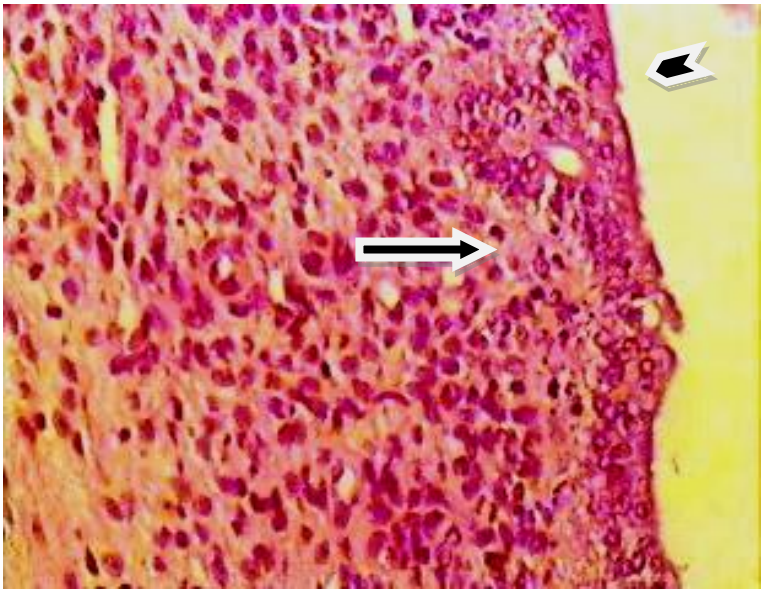


Figure 9. Stained uterine section of a dexamethasone treated adult mouse. Lowered Endometrial epithelium (arrow head), inactive proliferation of the endometrial stroma (arrow) and absence of active endometrial glands are characteristic features. (H – E, X 400).

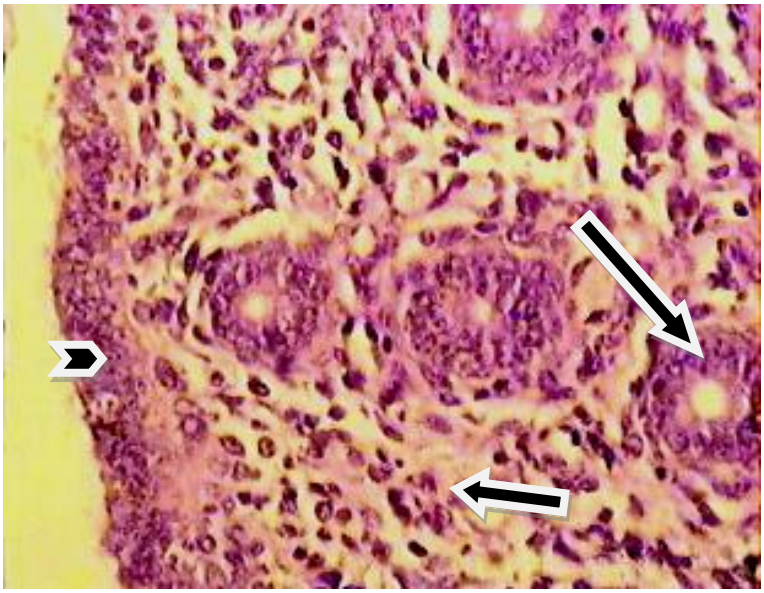


Figure 10. Positive response of the endometrial epithelium (arrow head), stromal cells (short arrow) and uterine glands (long arrow) in uterine section of a dexamethasone followed by gonadotropins treated adult mouse. (H – E, X 400).

استجابة صفات تركيبية – وظيفية لهورمونات محفزة للغدد الجنسية في مبايض و أرحام فئران بالغة معالجة بالديكساميثازون

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الخلاصـــة

استخدمت فئران سويسرية بيضاء بالغة منتظمة دورة الشبق ، قاربت أعمارها من الثلاثة شهور، لدراسة إمكانية استخدام النشاط الهرموني المحفز لتطور و نمو الجريبات المبيضية و النشاط الهرموني المحفز للإباضة و تكوين الجسم الأصفر و ذلك لعكس التغيرات المحتملة في الصفات التركيبية – الوظيفية نتيجة لحقن هورمون الديكساميثازون المصنع شائع الاستعمال لفاعليته القشرانية- السكرية..

أظهر الاختبار الإحصائي حصول زيادة معنوية في معدل الأوزان النسبية للمبايض و الأرحام مجتمعة للمجموعة المعالجة بالهورمونين المحفزين للغدة الجنسية. تميزت مجموعة الديكساميثازون بالانخفاض الملموس في الأوزان النسبية مقارنة بما يقابلها في المجاميع الأخرى. كان لحقن الهورمونين المحفزين للغدد الجنسية في الإناث التي سبق حقنها بالديكساميثازون تأثير إيجابي ملموس على معدل الأوزان النسبية للمبايض و الأرحام عند مقارنتها مع ما يقابلها في مجموعة السيطرة و المجموعة المحقونة بالديكساميثازون ، إلا أن المعدل كان منخفضا بمعنوية إحصائية عند مقارنته مع ما يقابله في مجموعة السيطرة المحقونة بالمحلول الفسلجي الطبيعي. استنادا على هذه النتائج المتمثلة بالأوزان النسبية التي تعكس مجمل الأحداث التركيبية الكيماوية الوظيفية في المبايض و الأرحام ، فإن حقن الهورمونين المحفزين للغدد الجنسية بالجرعات المستخدمة و مواعيد الحقن - خلال أربعة أيام من حقن الحيوانات بجرعات فترة الأيام الستة من الديكساميثازون، الفترة التي غطت أكثر من فترة دورة شبقية واحدة – كان قادراً الى حد ملموس على التعامل بإيجابية مع التأثيرات التثبيطية للهورمون القشراني السكري.

كان للفحص المجهري لشرائح المبايض و الأرحام مؤشرات إيجابية باتجاه قدرة حقنتي الهورمون المحفز للجريبات و حقنة الهورمون اللوتيني خلال فترة أربعة أيام – تغطي فترة دورة شبقية واحدة – على التعامل مع التغيرات التي تسببت بها الجرعات اليومية لعقار الديكساميثازون

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خلال فترة الأيام الستة و عكسها الى مدى ملموس أظهرته الصفات التركيبية الوظيفية. تسبب عقار الديكساميثازون في تثبيط واضح في تطور و نمو الجريبات و الأجسام الصفراء. أظهرت المكونات التركيبية الوظيفية للمبايض نموا و تطوراً إيجابيا نتيجة للمعالجة بالهورمونين المحفزين للمبايض بعد انتهاء جرعات الديكساميثازون مباشرة. مع ذلك فإن التحفيز الذي تسببت به جرعات الهورمونين المحفزين لم يصل الى المستوى الذي لوحظ في المجموعة التي حقنت على مدى ستة أيام بالمحلول الملحي الفسلجي الطبيعي. إن مرور أربعة أيام على آخر جرعة من الديكساميثازون حقنت الفئران خلالها بالمحلول الفسلجي الطبيعي لم تكن كافية للمبايض لتستعيد قدرتها على العودة الى النشاط التركيبي الوظيفي مقارنة مع ما يقابلها في حيوانات مجموعة السيطرة.

تميزت الصفات التركيبية الوظيفية التي شوهدت في الشرائح النسيجية للأرحام بتوجه يتماشى مع ما تم تسجيله في المبايض. تسبب الديكساميثازون في تثبيط نمو و تطور عناصر البطانة الرحمية و قد تمكن الهورمونين المحفزين للغدد الجنسية من عكس التثبيط و استعادة الصفات التركيبية الوظيفية للأرحام المعالجة بالديكساميثازون ، و لو أنها لم تصل الى المستوى الذي يمكن لهذين الهورمونين المحفزين أن يحدثاه من تغيرات إيجابية في الأرحام التي عولجت بالمحلول الملحي الطبيعي بدلا من الديكساميثازون. لم تكن فترة الأيام الأربعة التي أعقبت العلاج بالديكساميثازون كافية للأرحام للعودة الى مواصفاتها التركيبية الوظيفية الطبيعية. تمت مناقشة النتائج استنادا الى الأسس المتوقعة من تأثيرات مباشرة و غير مباشرة للهورمونات التي تم استخدامها على مكونات محور تحت المهاد – النخامية – المبيض – الرحم.