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Original Research Article

Some reproductive hormones in relation to ovarian activity in rats

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ABSTRACT

The present study is an endeavor to explore the relationship between induced hormonal alterations and ovarian activity in female rats. Fifty mature cycling female Albino rats were left for one week for acclimatization and offered balanced diet and water ad libitum. Animals were daily examined by vaginal smears to determine regularity of the estrous cycle. Rats were equally divided into 5 groups; control, hyperglycemic, hypoglycemic, hypercorticosteroid and hypocorticosteroid. At the end of 3 cycles, individual sera were obtained to determine glucose, malondialdehyde (MDA), estradiol and progesterone levels. Moreover, tissue specimens of the ovaries and the Fallopian tube were taken for histopathological examination. Results showed that all hormonal treatments induced alterations in the cellular characteristics of the cycle. Gonadosomaic index (GSI) did not show any remarkable variation. Alloxan or insulin treatments affected significantly serum glucose level in rats as. On the other side, hypercorticism led to marked elevation of glucose while hypocorticism showed no significant effect. Serum MDA showed significant elevation only in hyperglycemic and hypocorticoid groups. Hypoglycemia led to a significant decrease in serum estradiol while other treatments had no effect. Upon progesterone, hypoglycemia resulted in an increased level of the hormone while other treatments led to reduced levels of the hormone.

It was evident that abnormalities of the adrenal corticosteroids and / or pancreatic insulin levels are concomitant with irregular estrous cycle as well as ovarian and Fallopian tube alterations which led to deviated gonadal folliculogenesis and steroidogenesis.

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1. Introduction

The secretion of hypothalamic GnRH and pituitary FSH and LH, in a given moment is regulated by a neuroendocrine integrative mechanism that depends on the levels of ovarian steroids produced by the ovaries **(de La Iglesia and Schwartz, 2006)**. Also, **Nowland et al. (2011)** added that in rodents, corticosteroids are one of the neuroendocrine mediators that regulate the estrous cycle and therefore synergism among hypothalamic–pituitary–adrenal hormones is indispensable for optimum reproductive performance.Estradiol (E2) and progesterone (P4) play a crucial role in the female reproductive system. Together they act on the hypothalamus,pituitary and ovaries to regulate reproduction **(Conneely, 2001)**. This process depends on preovulatory E2 secretions **(Levine, 1997)**. Also, E2 and P4 have profound influence on neural circuits that regulate sexual behavior. **Yakubu et al. (2015)** concluded that deprivation of the female animal from normal progesterone level led to the appearance of polycystic ovarian syndrome (PCOS) and infertility. **Shepherd (2001)** showed that ovarian steroids control enzymes responsible for the protection of rat cells against oxidative damage caused by free oxygen radicals.

In experimental animals, the most commonly used chemical agent to induce type I hyperglycaemia is alloxan. while insulin is used to depress glucose level **(Esmerino et al., 1998)**. corticosteroids are considered the key regulators of whole-body homeostasis **(McKay and Cidlowski, 2003)**. In rats, **Hodges and Jones (1962)** induced hypercorticism using sc and ip injections with cortisol. **Reul et al. (1987)** induced hypercorticism by injection of dexamethasone and noticed that it resulted in low basal plasma concentrations of corticosterone and prevented the circadian-driven rise in circulating corticosterone. Upon glucose level, **Melmed et al.** (**2011)** showed that prolonged or high dose administration of corticosteroids was the most common cause of drug-induced diabetes mellitus(steroid-induced diabetes). In such case, the fasting blood glucose was ≥ 126 mg/dl and glycatedhaemoglobin"HbA1c " was ≥6.5%.The severity of steroid application variedat a wide range of doses,nature of the corticosteroid and route of administration.However, low glucocorticoid concentrations led to hypoglycemia and hypersensitivity to insulin. In the present study, induction of hypocorticism was implemented using surgical unilateral adrenalectomywhich was previously done in rats by **Hu et al. (2012)**. In this concern, **Nakayama et al. (1993)** and **Ibrahim et al. (2015)** induced unilateral adrenalectomy in rats and found, after 2 weeks, that the adrenal glands may substitute each other as all studied blood parameters were similar to those of the intact controls. **Debillon et al. (2015)** found that unilateral adrenalectomy led to an improvement in insulin resistance, diabetes mellitus and HbA1C. The authors concluded that either unilateral adrenalectomy or small doses of corticosteroids may result in suppressive influence on glucose level and insulin resistance.

Hormonal defects were found to be intimately related to infertility.**Vomachka and Johnson (1982)**and **Garris (1984)** reported that diabetes is linked to reproductive problems as impaired folliculogenesis, steroidogenesisand anovulation. **Garris et al. (1985)** found that the ovaries of diabetic mice exhibited stromal and follicular degeneration and a decline in the population of viable follicles with follicular atrophy and lowered serum estradiol and progesterone. **Valdes et al. (1991)** recorded that hyperglycaemia in ratsattenuated pituitary response to GnRH. In case of insulin-induced acute hypoglycemia, **Goubillon et al. (1996)** showed that it inhibited pituitary LH secretion in female animals. **Li et al. (2004)** reported that hormonal stress due to hyper or hypoglycemia stimulates the release of adrenal corticosteroids that suppress the activity of the hypothalamic GnRH pulse generator resulting in ovulatory and reproductive dysfunction. These results emphasized that hypothalamic pituitary adrenal (HPA) axis is

implicated in stress-induced suppression of the reproductive axis due to rise in circulating levels of corticosteroids. **Saltiel and Khan (2001)** noticed that during diabetes, tissues as muscle, fat and liver become less responsive or resistant to insulin. This state was also concomitant with polycystic ovarian disease (PCO), hypertension and atherosclerosis as well as reduced fertility due to hypogonadism. **Chabrolle et al. (2008)** reported that hormonal alterations either of the pancreatic islets or adrenal cortex were able to induce deleterious effects on reproduction. Also, glucose level abnormalities would reflect on ovarian activity **(Khowailed et al. 2012** and **Zhang et al., 2012)**.The authors added that withdrawal of gonadotropins resulted in a marked increase in granulosa cell apoptosis and inhibited E2and progesterone levels. **Jamilet al. (2013)** concluded that hormonal alterationsaffect the uterus structure and play a significant role in reproductive difficulties. Also, **Wallner et al. (1986)** showed that adrenal steroids can induce hypopituitarism and impaired gonadal functions resulting in infertility. In addition, **Nevagi and Kaliwwal (2001)** documented A decline in the reproductive capacity in animals with hypo or hyperfunction of adrenals.

Material and methods

A) Animals *:*

Fifty mature cycling Albino rats (160–180g B.W.) received from Animal Experimental Unit, Department of Physiology, Faculty of Veterinary Medicine, BeniSuef University were used in the present study. Rats were left for one week for acclimatization and kept under constant hygienic conditions as well as offered balanced diet and water ad libitum. Animals were daily examined, in the morning, through application of vaginal smears **(Marcondes et al., 2002)**, to determine regularity index of cycles and those showing 2 successive regular cycles were used. Rats were equally divided randomly into 5 groups; control, hyperglycemic, hypoglycemic, hypercorticosteroid and hypocorticosteroid groups. Moreover, vaginal smears, every morning, continued along the experimental period..

B) Experimental period :

The experimental period extended for 3 successive cycles **(Karkanias et al., 1997)**. Samples were collected also on the presumed estrous day following the experimental period to avoid the effect of estrous cycle phases on the estimated parameters. Moreover, animals were fasted for 2 hours before collection of blood samples **(Fantin et al., 2000)**.

C)Alloxan purification:

Before injection of alloxan (alloxan hydrate, Oxford Lab. Chem., India) into rats, purification of alloxan from impurities was done **(Hamdy, 1977)**.

D) Induction of hyper and hypoglycemia:

Induction of hyperglycemia was done by a single intraperitoneal injection of purified alloxan (150 mg / kg B.W.) in 1.0 ml saline and prepared freshly just before injection as reported by **Katsumata et al. (1993)**, while hypoglycemia was induced using repeated daily S/C doses of 1.0 U. insulin (mixtard, novo nordisk, Denmark) / 100 g B.W contained in 0.5 ml saline **(Li et al., 2004)**. After alloxan or insulin administration, rats with serum glucose levels of $\geq$180 or $\leq$ 90 mg/dl, respectively were included in the present study.

E) Induction of hyper and hypocorticism:

Hypercorticism was done by daily I/M injection of corticosteroids (dexamethasone sodium phosphate, Amriya for Pharmaceutical Industries, Alexandria, Egypt) at a dose rate of 2.0 mg/rat in 0.5 ml saline **(Saad et al., 1993)** along the experimental period while, hypocorticism was carried out after surgical unilateral adrenalectomy**(Ali et al., 2003)**.

F) Samples collection :

At the end of experiment, individual blood samples were collected, from the medial canthus of the eye into sterile tubes and sera were preserved at -20°C for determination of biochemical parameters. Moreover, tissue specimens, including the ovaries (following calculation of GSI) and the middle third part of

the Fallopian tube (secretory part) were taken out and preserved separately in 10 % formalin for histopathological examination.

G) Calculation of the gonadosomatic index (GSI) :

The ovaries were removed and weighed separately in a closed vial to recordthe mean ovarian weight. The gonadosomatic index was calculated from thefollowing formula as mentioned by**Adebiyi (2013)**.

$$GSI = \frac{\text{Weight of gonads}}{\text{Total bodyweight}} \times 100$$

H)Quatitative determination of glucose :

Special glucose kits (Spinreact, Spain), at 505 nm wavelength, were used todetermine serum glucose content as outlined by **Burtis and Bruns(1999)**.

I) Estimation of lipid peroxidation ″malondialdehyde″ content (MDA) :

Serum lipid peroxidation was estimated colorimetrically (**Albro et al., 1986)** using photoelectric colorimeter (spekol 11, Germany) at 530 nm.

J) Determination of serum estradiol (E2) and progesterone :

Serum estradiol and progesterone were estimated by competitive ELISA technique. For both hormones, Cayman's ELISA Kits (USA) were used as outlined by **Maxey et al. (1992)**. The intensity of the color was determined spectrophotometrically (MiniVidas, France) at 412 nm.

K)Histopathological studies:

Samples of the ovaries and Fallopian tubes were fixed in 10% formalin , embedded in paraffin then stained by haematoxylin and eosin "H and E" **(Drury and Wallington, 1980)** and used for histopathological studies as outlined by **Jones and Hunt (1983)**.

L) Statistical analysis of the data:

The obtained data were subjected to the statistical analysis as outlined by **Snedecor and Cochran (1987)** as well as **SAS Program (1994)**.

Results

All females, before different treatments, showed 2 successive regular estrous cycles. All treatments induced alterations in the rhythmicity and phasic cellular characteristics of cycles. Table 1 disclosed that GSI of rats after hormonal alterations did not show any remarkable variation. Also,alloxan or insulin affected significantly serum glucose level.Hypercorticism led to marked elevation of serum glucose while hypocorticism showed no significant effect on glucose level.SerumMDA showed significant rise in hyperglycemic and hypocorticoid groups. E_2 level in hyperglycemic, hypercorticoid and hypocorticoid treatments did not differ than control group, while induced hypoglycemia led to significant decrease in E_2. Hypoglycemia resulted in remarkable increase in progesterone while the other treatments were concomitant with significant decreased levels.Alloxan-treated group exhibited ovaries with multiple cystic follicles accompanied with proliferation of fibrous connective tissue and angiogenesis with necrosis of some glandular epithelium of the Fallopian tube. In hypoglycemic group, the ovaries showed hyperplasia of a number of corpora lutea and reduced number of growing ovarian follicles while the Fallopian tube showed congestion of blood vessels and capillaries with vacuolation of cytoplasm of the lining glandular cells. Ovary from corticosteroids-treated group showed multiple cysts of Graffian follicles; most of them is lined by one layer of flattened follicular epithelium with increased number of corpora lutea while Fallopian tube showed proliferation of fibrous tissue and reduced number of glands. Ovary from hypocorticoid rats revealed varying stages of ovarian follicles at different stages of growth; most of them is lined by stratified follicular epithelium with condensation of the stroma forming the theca and the Fallopian tube

exhibited congestion and dilatation of the majority of blood vessels.

Discussion

Results of the present study clarify that alloxan was able to induce type I hyperglycemia; a result that confirms previous report **Rohilla and Ali (2012)** who stated that alloxancauses selective necrosis of β cells and generates reactive oxygen species (ROS) that affect DNA of β cells resulting in DNA fragmentation and cell damage. **Li et al. (2004)** found that administration of insulin into female rats lowers glucose level as it increases the rate of glucose transport across the cell membrane. In the present study, hypercorticism was implemented by using daily i.m. injection of a corticosteroid**(Reul et al. 1987)**. On the other side, induction of hypocorticism was done by surgical unilateral adrenalectomy which was previously used in rats by **Ibrahim et al. (2015)**.It appears that all induced hormonal alterations did not have influence on either the body weight or the GSI.In this concern, the previous study of **Jamil et al. (2013)** found that in hyperglycemic rats, the body and uterus weights were decreased while no significant difference was seen between control and insulin-treated groups.Therefore, in conclusion, it seems that glucose level as well as the period of hormonal alteration have the decisive role to affect the body weight and the GSI. In the present study, the experimental period was only for 12 days; a short period which was not sufficient to affect either body weight or the GSI.Table 1 shows that alloxan induced hyperglycemia while insulin was potent to lower glucose level as compared to the control value. These results emphasizes reports of **Li et al. (2004)** and **Rohilla and Ali (2012).** Moreover, the table clarifies that administration of corticosteroids led to significant hyperglycemia while unilateral adrenalectomy had no effect on glucose level. These findings receive an evidence from previous records of **Merke and Bornstein (2005)** who showed that prolonged or high dose administration of corticosteroids was the most common cause of drug-induced diabetes (steroid diabetes). The severity of this side effect varies according to doses, nature of the applied corticosteroid and route of administration **(Melmed et al., 2011)**. On the other hand, the same authors recorded that low glucocorticoid concentrations led to hypoglycemia, decreased glycogen stores, and hypersensitivity to insulin. In the same sequence,**Ibrahim et al. (2015)** induced unilateral adrenalectomy in rats and found, after 2 weeks, that the adrenal glands may substitute each other as all studied blood parameters including glucose level were similar to those of the intact control group.

MDA is considered an important marker for elevation of ROS and oxidative stress ***(Farmer and Davoine, 2007)***. Serum MDA showed significant elevation only in hyperglycemic and hypocorticoid groups. In this concern, **Limon-Pacheco and Gonsebatt (2009)** reported that high level of glucose causes energy stress for the cells and consequently a reduction of the steroid production as well as an imbalance between ROS and antioxidant defenses. The elevation of MDA in adrenalectomized animals may be explained due to the sudden withdrawal of corticosteroids from the circulation as these hormones have a protective mechanism against stress. **Ericson-Neilsenand Kaye (2014)** showed a quantitative relation between stress intensity of and corticoid dose to prevent this effect.

It was observed that all hormonal applications significantly induced alterations in the rhythmicity and phasic cellular characteristics of the estrous cycle. In the same sequence, the current study showed that hyperglycemia, hypercorticoid and hypocorticoid treatments were accompanied with insignificant variation in estradiol levels than that of the control group. On the other side, induced hypoglycemia led to significant decrease in the estradiol level. Regarding progesterone, hypoglycemia resulted in remarkable increase in that hormone while the

other treatments were concomitant with significant decreased levels of progesterone as compared with control value. These results emphasize a state of hormonal imbalance in sex steroids after induction of hormonal alterations in mature female rats which has an intimate relation with the irregularity of the estrous cycle.In this respect, **Garris et al. (1984)** showed that in female rats, either alloxan treatment or insulin effectively altered progesterone level and luteal functions as compared with controls. The authors also detected, in such cases, impaired ovarian steroid production with reproductive impairment. **Poretsky et al. (1988)** infemale rats found that the androstenedione to estrone ratio was significantly lowered in insulin group. **Nevagi and Kaliwwal (2001)** mentioned the importance of the adrenal corticoids for the optimal performance of the ovarian functions and reproductive efficiency. The authors recorded a decline in the reproductive capacity in animals with hypo or hyperfunctioning of adrenals. **Simardet al. (2005)** stated that high concentrations of glucose may reduce progesterone secretion in rat. **Chakrabartyet al. (2006)** concluded that hyperinsulinemia plays an important role in the pathogenesis of polycystic ovary syndrome (PCOS) in rats. Hyperinsulinemic animals had erratic estrous cycles, with prolonged metestrus-diestrus or diestrus-proestrus stages, significantly decreased levels of serum progesterone, and significantly prematurely luteinized ovarian follicles with prominent thecal and interfollicular stromal proliferation. The authors concluded that hyperinsulinemia in rats causes hormonal and ovarian changes similar to those in women with PCOS. **Chabrolle et al. (2008)** recorded reproductive dysfunction in the diabetic female rats associated with altered folliculogenesis and steroidogenesis. **Gupta and Bhatia (2008)** reported that hypercorticism has a suppressive effect on endogenous steroid production due to the influence on hypothalamus-pituitary axis. **Khowailed et al. (2012)** reported that in female rats diabetes is associated with increased risk of reproductive problems as impaired folliculogenesis and steroidogenesis and anovulation. **Inhasz Kiss et al. (2013)** concluded that mild hyperglycemia did not impair gonadotropin levels or estrous cylicity but ovarian steroid concentrations were altered. The authors showed that induced glucose level alterations were associated with changes in the ovarian steroid hormone levels. In addition, **Jamil et al. (2013)** found that insulin deficiency and oxidative stress affect the uterus structure which might play a significant role in the reproductive difficulties observed during hyperglycemia. **Nagatani et al. (2013)** reported that glucose availability controls reproductive activity through modulation of LH secretion. Therefore, the aforementioned series of studies emphasizes that deviations of either adrenal steroids or pancreatic hormones have an intimate relation with alterations of gonadal steroids and consequently regularity of the estrous cycle and reproductive performance.

Looking at the histopathology of ovaries and Fallopian tubes, it is obvious that ovaries of alloxan group exhibited multiple cystic ovarian follicles concomitant in the Fallopian tube with proliferation of fibrous connective tissue, angiogenesis and necrosis of glandular epithelium. On the other side, ovary from insulin-treated group disclosed hyperplasia of a number of corpora lutea and reduced number of growing ovarian follicles while in the Fallopian tube, there was congestion of most blood vessels and capillaries with vacuolation of the cytoplasm of the lining glandular cells.Ovary from corticosteroids group showed multiple cysts of Graffian follicles with increased number of corpora lutea while the Fallopian tube showed proliferated fibrous tissue with reduced number of glands. The ovary from hypocorticoidrats revealed varying stages of ovarian follicles at different stages of growth with condensation of stroma forming the theca while the Fallopian tubes showed congestion and dilatation of the majority of blood vessels.

These findings clarify ovarian and Fallopian tube abnormalities concomitant with induced hormonal alterations.

Thus, it could be concluded that reproductive ovarian hormones are not only affected by pituitary gonadotropins but also could be deviated by alterations in circulating levels of pancreatic insulin and adrenal corticosteroids which increase the level of ROS leading to changes in ovarian and Fallopian tube structures and consequently regularity of the estrous cycle.Therefore, the present study recommends the importance of evaluating adrenal corticosteroids and pancreatic insulin hormone levels as a probable cause of ovarian dysfunction and consequently infertility among animals.

Table 1: Serum parameters of mature female rats after different hormonal treatments (Mean ± SE).

Item/ Groups	Control	Hyperglyc	Hypoglyc	Hypercort	Hypocort
GSI %	23.92 ± 4.06	23.40 ± 3.87	23.35 ± 2.18	24.30 ± 4.08	23.80 ±4.22
Glucose (mg %)	104.04 ± 5.06	261.94 ±9.54*	76.51 ±4.87*	183.22 ±8.91	99.79 ±7.74
MDA nmol/ ml	0.25 ±0.02	0.33 ±0.03*	0.27 ± 0.04	0.28 ±0.05	0.35 ±0.03*
Estradiol pg / ml	42.09 ±5.84	39.19 ± 3.54	18.64 ±2.06*	31.07 ±3.68	28.94 ±4.49
Progest ng / ml	9.17 ±1.05	5.54 ±0.76*	14.23 ±1.76*	5.89 ±0.42*	4.77 ±0.85*

In the same row, values with *differ significantly from control group at $P \leq 0.025$.

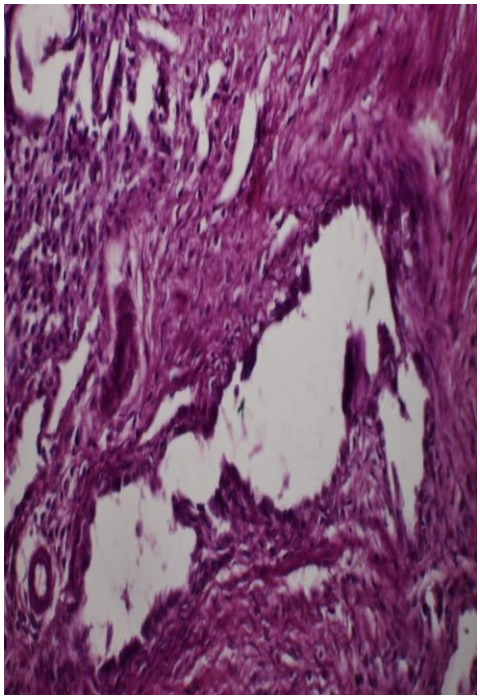

Fig. 1 : Section of Fallopian tube from alloxan-treated group showing proliferation of fibrous connective tissue and angiogenesis (new capillaries formation)
with necrosis of some glandular epithelium (H & E, mm 400).

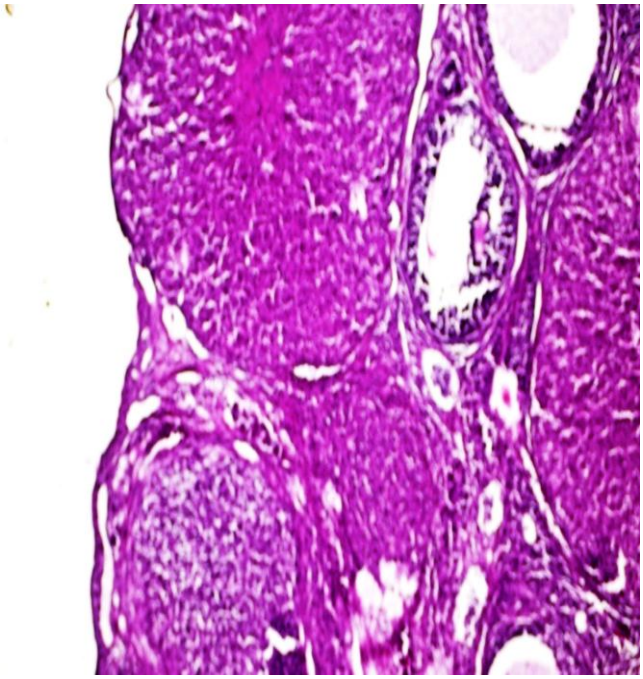

Fig. 2 : Section of ovary from insulin-treated group disclosing hyperplasia of a number of corpora lutea and reduced number of growing ovarian follicles (H & E, mm 200).

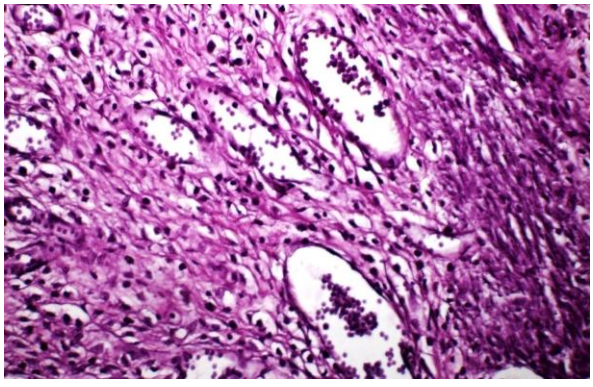

Fig. 3 : Section of Fallopian tube from insulin-treated group revealing congestion of most blood vessels and capillaries with vacuolation of the cytoplasm of the lining glandular cells (H & E, mm 400).

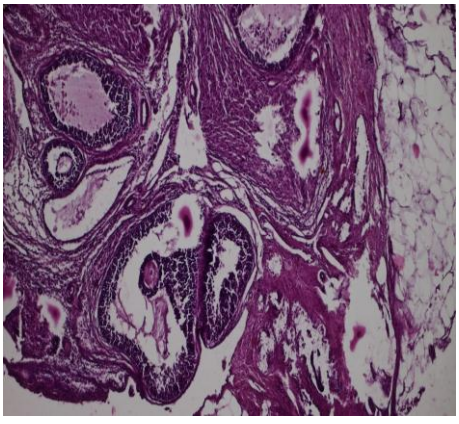

Fig. 4 : Ovary from corticosteroids-treated group showing multiple cysts of graffian follicles; most of them is lined by one layer of flattened follicularepithelium with increased number of corpora lutea (H & E, mm 100).

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