Egypt. J. Anat. Vol. 26 (1) 1-35, January 2003

ISSN 1110-2144

INFLUENCE OF HYPERPROLACTINEMIA INDUCED EXPERIMENTALLY ON THE HYPOTHALAMO-HYPOPHYSIAL GONADAL AXIS IN ADULT FEMALE ALBINO RATS

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INTRODUCTION

Prolactin secretion by the pituitary gland is under predominantly negative control by the hypothalamus (**Leong et al., 1983**). A physiological rise in plasma prolactin level occurs during pregnancy and early in lactation. The introduction of the assay of prolactin in human serum during 1970, resulted in the definition of hyperprolactinemia syndrome i.e. menstrual disturbance, infertility and galactorrhea in women. The principal modes of presentation of hyperprolactinemia in women are infertility and menstrual disorders (**Guanasthe, 1993**).

Most of the previous works have been carried out to investigate the pathophysiological aspect of hyperprolactinemia associated infertility. However, little attention has been paid to the histopathological changes that occur in the female reproductive system in such circumstances.

From the available literature, no histological studies dealing with the effect of experimentally-induced hyperprolactinemia on the neurons of the hypothalamic nuclei or the pituitary gland were previously done. Varied literature supported the view that the arcuate nucleus provides the final common neurosecretory pathway over which the C.N.S controls the anterior pituitary gland (**Everitt et al., 1986**). Effects of gonadal steroids on the hypothalamus are ultimately translated into alterations in the pituitary secretory activity with subsequent change in the endocrine gland which are dependent on pituitary hormones (**Farnworth, 1995**).

Sulpiride is a potent antidopaminergic agent with antipsychotic and antidepressant activity (**Serra et al., 1990**). It acts on prolactin and gonadotrophic secretion.

Therefore, this work was carried out to study the changes that would occur in response to experimentally induced hyperprolactinemia using sulpiride in the hypothalamo-hypophysial gonadal axis.

The results will be used to evaluate the deleterious effects of hyperprolactinemia towards further understanding the exact pathological changes involved in associated reproductive disorders.

The structure of arcuate nucleus of hypothalamus, gonadotrophs and mammotrophs of the pars distalis and the ovary in sulpiride induced hyperprolactinemic albino rats will be investigated in this work using histological, immunocytochemical as well as ultrastructural techniques.

MATERIAL AND METHODS

A total number of 80 adult cycling female Sprague Dawley rats (isolated for 15 days to exclude pregnancy) were used in this experiment. Vaginal smears were examined daily. Cytological preparations were stained with Shorr's technique (**Shorr, 1941**). Vaginal cornification was interpreted as a sign that ovulation has occurred or about to occur. Only rats showing at least two consecutive 4 days estrus cycle with proestrus, estrus, metestrus and diestrus (**Freeman, 1988**) were used. These animals were maintained under standard light / dark cycles, fed normal rat chew ad libitum and allowed free access to water. The animals were divided into 4 groups (20 animals for each group); one control, one lactating and two experimental groups. The experimental groups received sulpiride in a dose of 100 mg / Kg in saline twice daily intraperitoneally for 2 and 4 weeks respectively. The dose was taken after **Perez and Lawzewitsch (1984)**.

Sulpiride N. (1-ethyl-2- pyrrolidinyl meth) -2 methoxy-5- sulfamoyl benzamide was obtained from Memphis company (Egypt) in the form of capsules, each one contained 50 mg of pure sulpiride. The powder was dissolved in saline solution (0.9% Nacl).

Specimens were taken from the hypothalamus at the level of median eminence for studying the arcuate nucleus, the pituitary gland and the ovary. These specimens were prepared for the light and electron microscopic examination.

For immunohistochemical technique mouse monoclonal antibodies were used to demonstrate both FSH and LH producing cells in pars distalis. Another antibody was used for demonstration of prolactin receptors inside both the arcuate nucleus and ovary. These antibodies were supplied as 20 µg / ml of antibody purified

from ascites fluid. A formalin fixed, paraffin blocks were used. Sectioning was done on special sialinized slides in a 5 μg thick (**Cerson, 1990**). The cellular localization after the usage of the 3 types of antibodies was cytoplasmic.

RESULTS

Hypothalamus (arcuate nucleus) :

Group I :

The arcuate nucleus is well localized as a neuronal collection on each side of the third ventricular recess (Fig. 1). Immunocytochemical technique for demonstration of prolactin receptors reveals positive reaction in the cytoplasm of the neurons (Fig. 2). Electron microscopic examination reveals light and dark neurons based on electron density of the cytoplasm, with predominence of the light one. The light neurons are rounded or fusiform in shape with rounded euchromatic nuclei and shallow indented nuclear envelope. Their cytoplasm is electron lucent and rich in organelles as Golgi bodies, mitochondria, free ribosomes, rER, as well as few lysosomes and lipofuscin bodies (Fig. 3). The dark neurons are characterized by electron dense cytoplasm with abundant free ribosomes. The nucleus is irregular with highly folded nuclear envelope (Fig. 4).

Group II :

Immunoreactivity for prolactin receptors reveals a more increase in intensity of the reaction in comparison with those of the control (Fig. 5). Ultrastructural examination of the arcuate nucleus of lactating animals shows an increase in the cytoplasmic organelles and irregularity in the nuclear envelope of neurons (Fig. 6).

Group III :

Electron microscopic examination of this group reveals the following features. The light neurons show a marked increase in the incidence of lysosome-like dense bodies and lipofuscin pigment. The nucleus has dissociated chromatin (Fig. 7). Some dark neurons have dilated Golgi bodies, multiple vacuoles, lysosome-like dense bodies and lipofuscin pigments (Fig. 8).

Group IV :

PRL receptors immunoreaction shows a reduction in the staining intensity in the neurons in comparison with those of the control (Fig. 9). Ultrastructurally, there

is a progressive increase in the dense bodies and lipofuscin contents. Large nematosomes (stigmoid bodies) are frequently detected in the cytoplasm (Fig. 10). The dark neurons show features similar to those of the previous group. However, some of these neurons show an increase in the electron density of both the nucleus and cytoplasm and irregularity of the nuclear outline.

Pars distalis :

Group I :

Immunocytochemical demonstration of FSH and LH cells shows some cells with central or eccentric nuclei and brown granules in the cytoplasm which represent FSH and LH in tissue (Figs. 11, 12). Ultrastructural examination clearly reveals five types of the chromophils. Mammotrophs and gonadotrophs which are studied in this work show the following features. Mammotrophs have elongated shape with rounded or oval nuclei. Their secretory granules are large, pleomorphic and electron dense. The cytoplasm is rich in organelles. Gonadotrophs are rounded or polyhedral and contain rounded secretory granules of variable sizes and electron density (Fig. 13).

Group II :

By immunocytochemistry, the intensity of the reaction in both FSH and LH cells is more or less similar to that in control. Electron microscopic examination reveals an increase in the amount of secretory granules of both mammotrophs and gonadotrophs (Fig. 14).

Group III :

Mammotrophs have some degree of activation. The cytoplasm contains Golgi bodies, rER and secretory granules. The nucleus has fine dispersed chromatin (Fig. 15). Gonadotrophs show large amount of secretory granules and well developed Golgi bodies. Mitochondria have variable degrees of destructed cristae (Fig. 16).

Group IV :

Immunocytochemical demonstration of FSH and LH cells shows a marked increase in the staining intensity in the cytoplasm of both cells (Figs. 17, 18).

Ultrastructurally, chromophils are hardly differentiated from each other. Some mammotrophs show dense nuclei with coarse clumps of chromatin. Others show whorely organization of highly developed rER and small amount of granules which

are of variable electron density. Some gonadotrophs show dark nuclei with coarse clumps of chromatin. The cytoplasm contains large number of secretory granules and dilated rER (Fig. 19). Most of gonadotrophs exihibit dense cytoplasm and nuclei. The cytoplasm contains aggregated dense secretory granules, large vacuoles and highly destructed vacuolated mitochondria. Other cells have electron lucent cytoplasm and rarified karyoplasm (Fig. 20).

Vaginal Smear :

The estrus cycle stages are determined by staining the vaginal smear by Shorr's stain. After examination of cellular changes in smears from vaginal epithelium of an untreated cyclic rats four stages can be detected : Diestrus stage : this stage is typically atrophic, consisting of very large number of polymorphs and some nucleated epithelial cells (Fig. 21). Proestrus stage : is characterized by striking decrease in the number of leukocytes. The smear is composed mainly of nucleated epithelial cells (Fig. 22). Estrus stage : is characterized by the presence of cornified cells. These cells are polygonal and usually lie flat and discrete (Fig. 23). Metestrus stage : cornified cells, leukocytes as well as nucleated epithelial cells are present in this stage (Fig. 24).

Vaginal smears of both physiological and induced hyperprolactinemic animals don't show cyclic changes. They show few scattered leukocytes and nucleated epithelial cells "lactational anestrus" (Fig. 25).

Ovary :

Group I :

Diestrus stage is characterized by multiple functionally regressing corpora lutea and corpora albicans. Multiple small growing follicles are also present (Fig. 26). Immunocytochemical demonstration of prolactin receptors reveals a moderate positive staining in the majority of the cells of both the corpus luteum and the stromal cells (Fig. 27).

Proestrus stage is characterized by preovulatory growth of follicles (Fig. 28). Prolactin receptors expression is more intense in the follicles of proestrus stage in comparison to that in diestrus stage (Fig. 29).

Estrus stage is characterized by mature graffian follicles (Fig. 30). Prolactin receptors expression is decreased in this stage in comparison to proestrus & diestrus stages (Fig. 31).

Metestrus stage is characterized by a large number of corpora lutea (Fig. 32). High prolactin receptor expression in metestrus ovary is observed (Fig. 33).

Group II :

Lactating ovary stained with H & E shows cystic follicles that are lined by a thin layer of small dark cells and may have cumulus oophorus cells that surround a resorbed ovum (Fig. 34). Other follicles are lined by several layers of follicular cells. Some of these cells have dark rounded nuclei. Others exfoliated in the lumen (Fig. 35).

Group III :

In H & E stained sections; there are multiple cystic follicles of variable sizes in the ovarian stroma (Fig. 36). Some follicles have neither ova nor zona pellucida and the follicular cells are disorganized (Fig. 37).

Group IV :

Examination of paraffin sections stained with H & E reveal that the ovaries of most of the animals are occupied by some large cystic follicles. Numerous atretic follicles, with detached ova are also present (Fig. 38).

Dense bodies surrounded by variable amounts of cytoplasm are shown in the lumen of these follicles. There are other dense bodies scattered among the follicular cells. These bodies have features similar to those of the apoptotic bodies (Fig. 39).

Immunohistochemical reaction for demonstration of prolactin receptors is variable from weakly positive in granulosa cells to negative in theca cells. However, stroma cells show moderately positive reaction (Fig. 40).

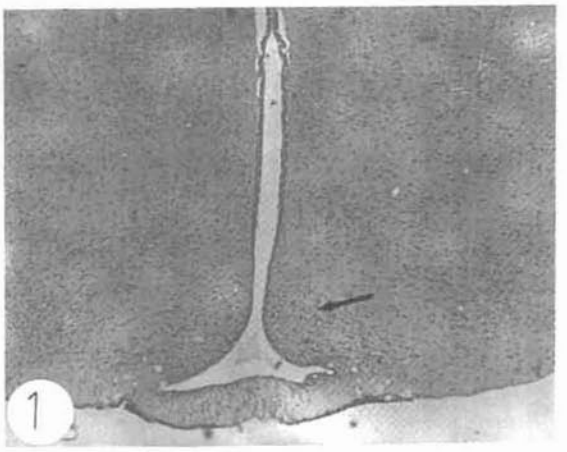


Fig. (1) : A photomicrograph of a frontal paraffin section in the brain at the level of the mediobasal hypothalamus. Note the arcuate nucleus (↑) and third ventricle (IIIV).

(Hx. & E.; x 40)

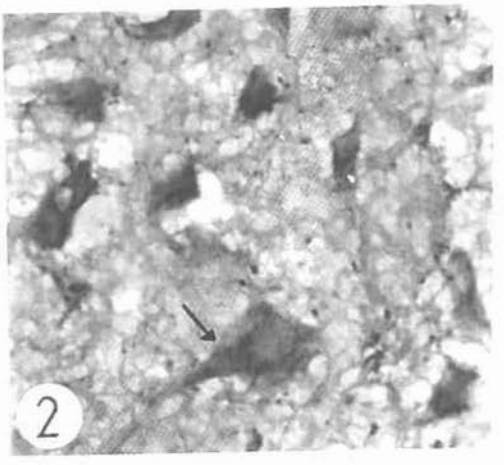


Fig. (2) : An immunohistochemical technique for demonstration of prolactin receptors in arcuate nucleus of group (I) showing: a positive reaction in the cytoplasm of some neurons (↑).

(x 1000)

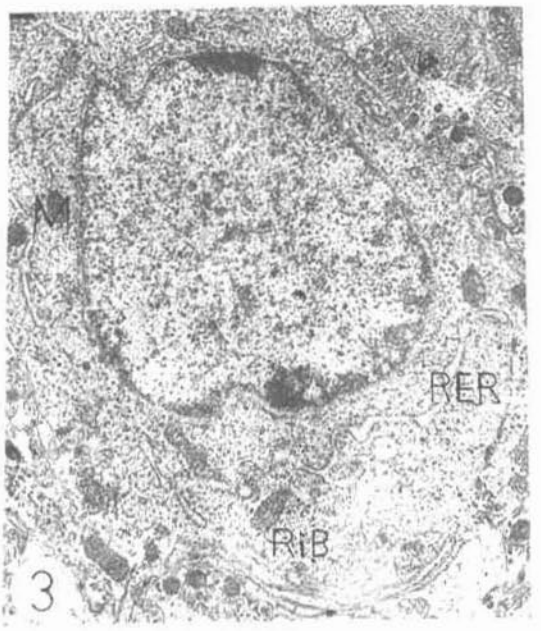


Fig. (3) : An electronmicrograph in the arcuate nucleus of control animals showing; a light neuron. It possesses a nearly rounded nucleus with clear cytoplasm rich in organelles as rough endoplasmic reticulum (RER); ribosomes (Rib), and mitochondria (M).

(x 5000)

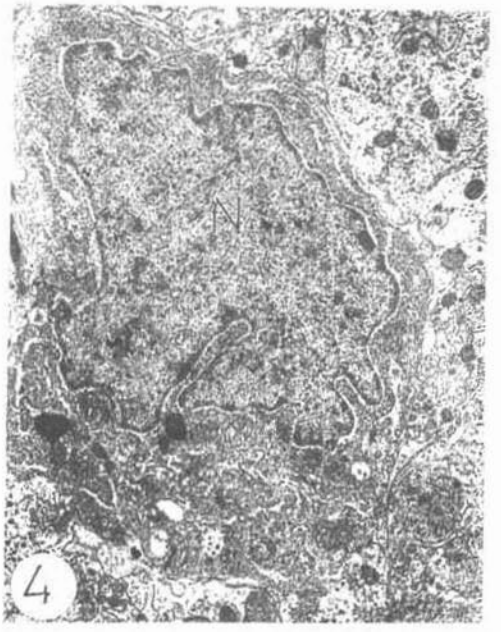


Fig. (4) : An electronmicrograph of a dark neuron in a control arcuate nucleus showing; both the cytoplasm and the nucleus are relatively dense compared with the light neuron. Note the irregular contour of the nucleus (N) and the cytoplasm.

(x 5000)

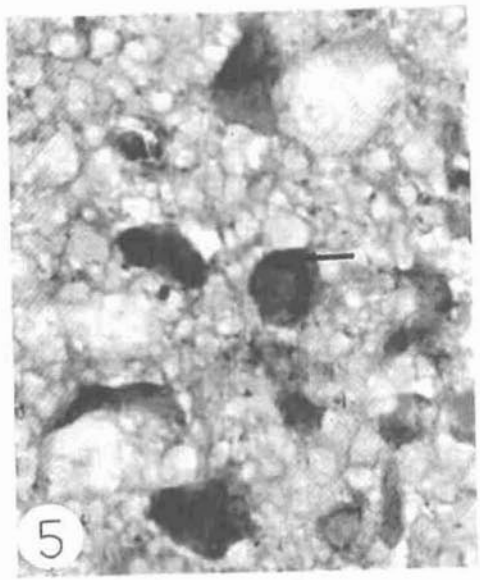


Fig. (5) : An immunocytochemical technique for demonstration of prolactin receptors in arcuate nucleus of group II animals showing, a highly positive reaction in comparison to those of control (↑).

(x 1000)

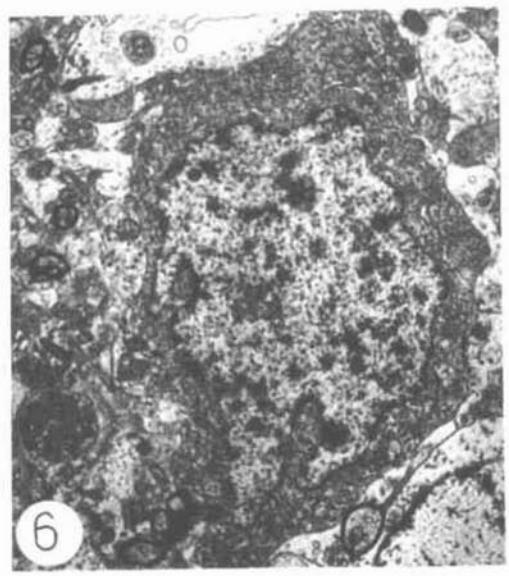


Fig. (6) : An electronmicrograph in the arcuate nucleus of group II animals, showing irregular outline of both nucleus and cytoplasm. The latter is rich in free ribosomes, RER, mitochondria and Golgi bodies.

(x 5000)

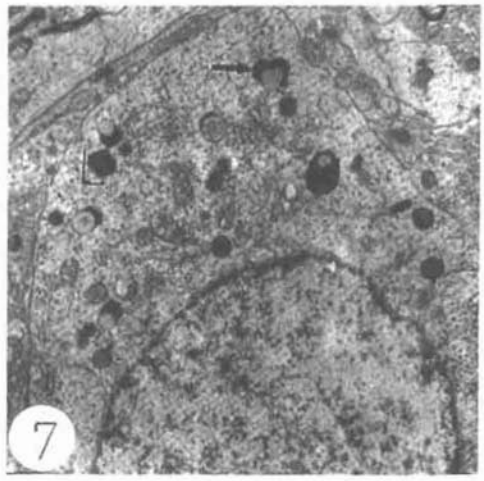


Fig. (7) : An electronmicrograph in the arcuate nucleus of group III animals showing, a light neuron with a large number of lipofuscin bodies (↑) and lysosome like dense bodies (L) in the cytoplasm.

(x 5000)

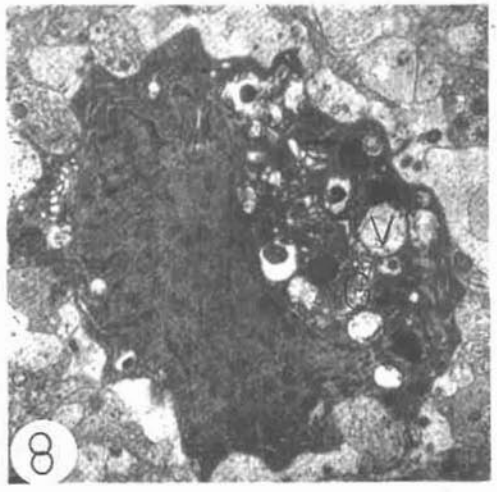


Fig. (8) : An electronmicrograph in the arcuate nucleus of group III animals, showing; a dark neuron. Note the dilated Golgi cisternae (G), multiple electron dense bodies (↑), lipofuscin bodies and multiple vacuoles (V) in the dark cytoplasm.

(x 5000)

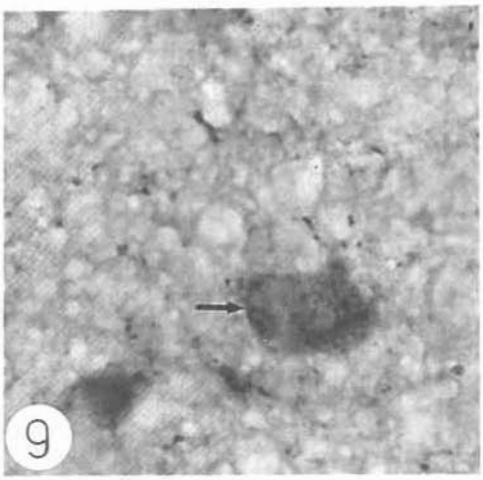


Fig. (9) : An immunohistochemical technique for demonstration of prolactin receptors in arcuate nucleus of group IV animals showing; weakly positive reaction in the neuron (↑) in comparison to those of the control.

(x 1000)

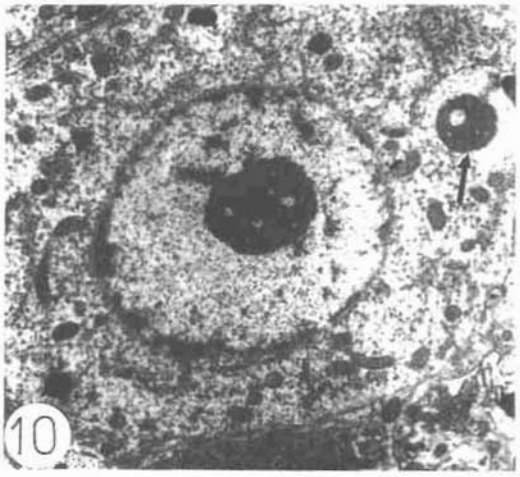


Fig. (10) : An electronmicrograph in the arcuate nucleus of group IV animals showing; a light neuron with well defined vesicular nucleus. Note the presence of nematosome (↑) (stigmoid body) in the cytoplasm.

(x 5000)

Fig. (11) : An immunohistochemical technique for demonstration of FSH in the pars distalis of group I animals. It shows a moderate positive staining of the cytoplasm of some cells.

(x 1000)

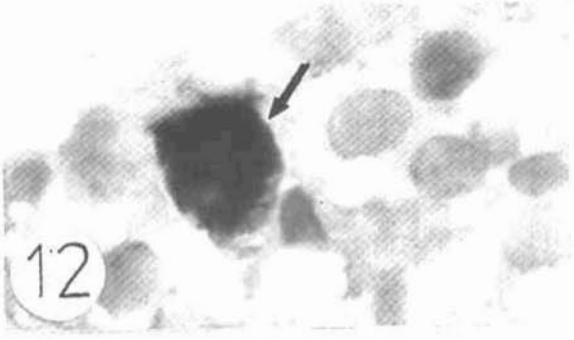


Fig. (12) : An immunohistochemical technique for demonstration of LH in the pars distalis of group I animals showing; a positive reaction in the cytoplasm of a cell (↑) among the other negative cells.

(x 1000)

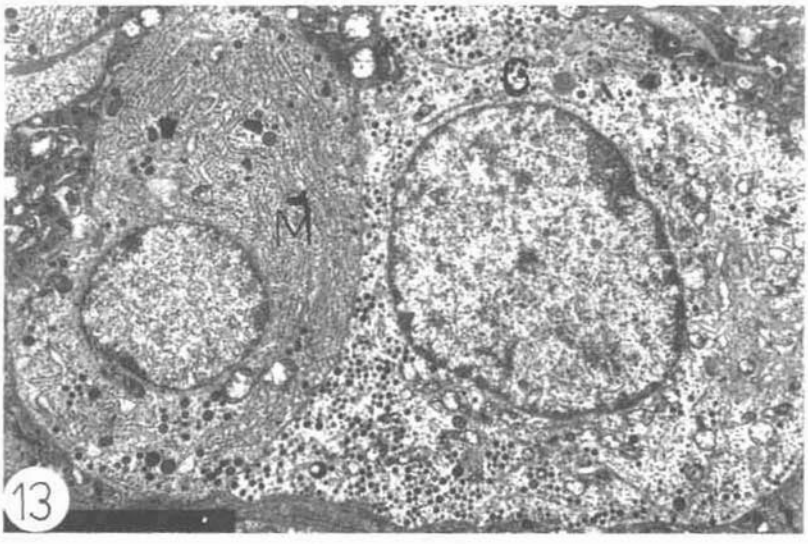


Fig. (13) : An electronmicrograph in the pars distalis of group I animals showing two adjacent cells. The right one is a mammotroph (M). It is oval in shape with characteristic large sized electron dense secretory granules, strands of RER, mitochondria and Golgi bodies. The left one is a gonadotroph (G) containing secretory granules of variable sizes and electron density.

(x 4000)

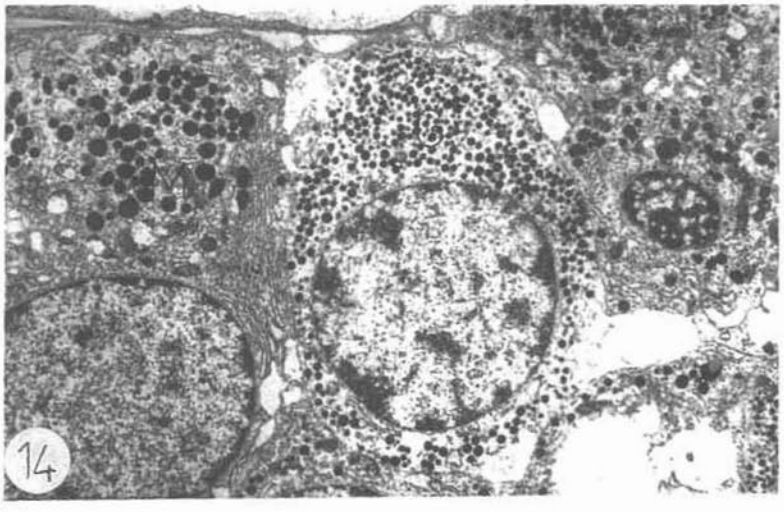


Fig. (14) : An electronmicrograph in the pars distalis of group II animals. It shows, a gonadotroph (G) and on adjacent mammotroph (M). Note the marked increase in amount of secretory granules in both cells. There is also a mild dilatation in RER in both cells.

(x 4000)

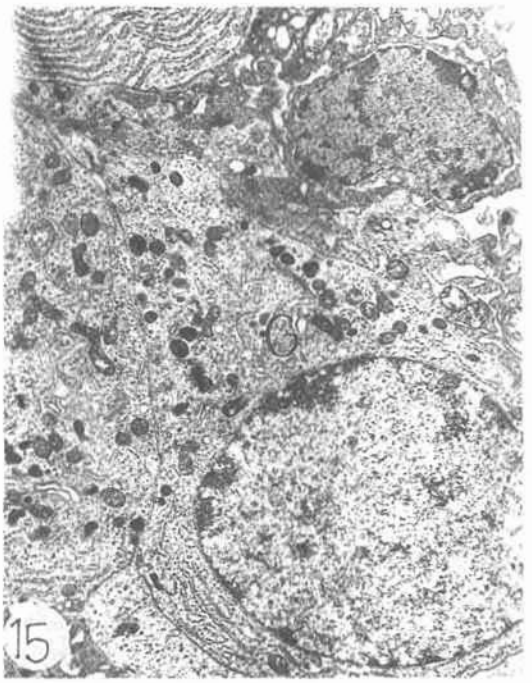
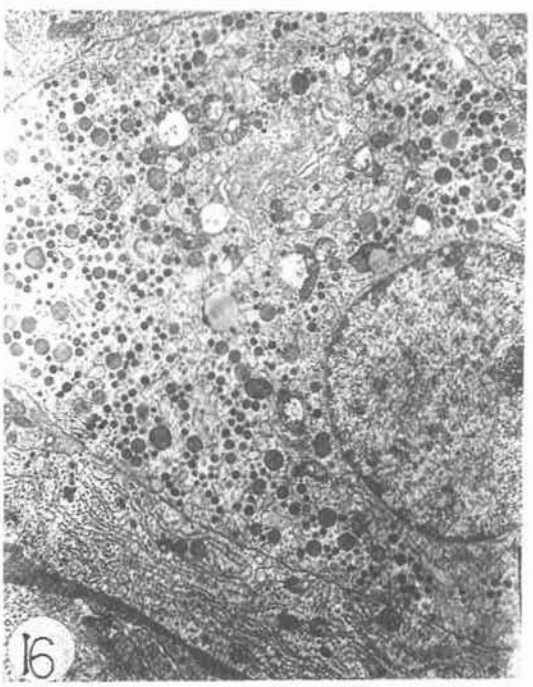


Fig. (15) : An electronmicrograph in the pars distalis of group III animals showing a gonadotroph. The cytoplasm is occupied by a large number of secretory granules of variable size and electron density, Golgi body and strands of RER

(x 4000)

Fig. (16) : An electronmicrograph in the pars distalis of group III animals showing a gonadotroph. The cytoplasm is occupied by a large number of secretory granules of variable size and electron density, Golgi body and strands of RER.

(x 4000)



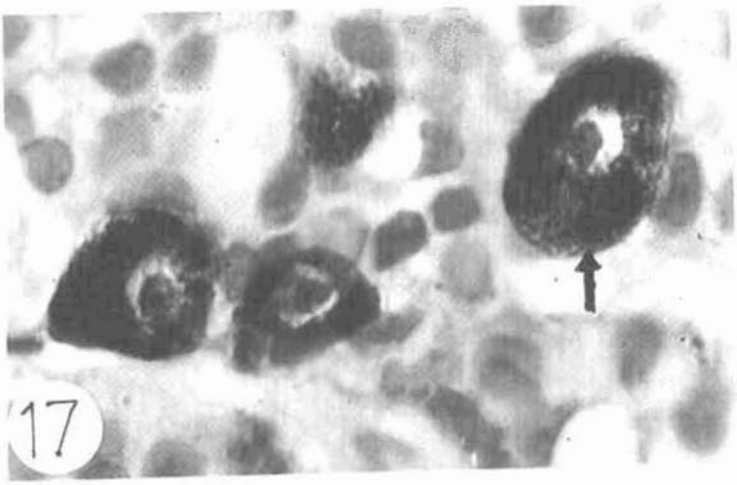


Fig. (17) : An immunohistochemical technique for demonstration of FSH in the pars distalis of group IV animals showing a highly positive staining in the cytoplasm of some cells (↑).

(x 1000)

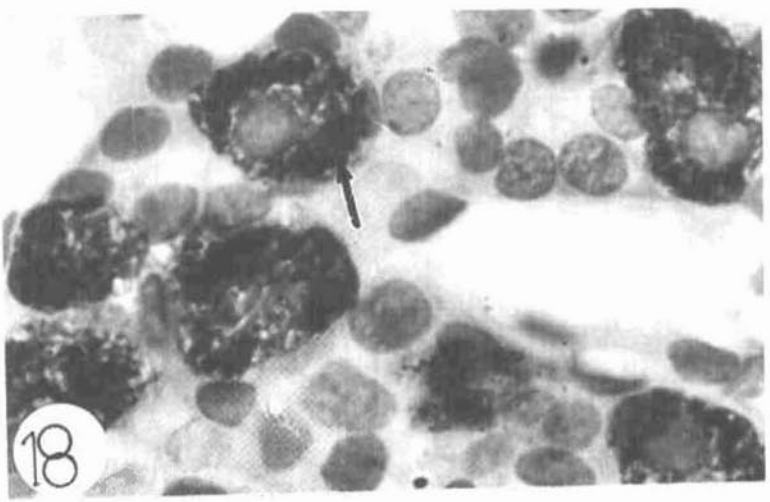


Fig. (18) : An immunohistochemical technique for demonstration of LH in the pars distalis of group IV animals showing a highly positive staining in the cytoplasm of multiple cells (↑).

(x 1000)

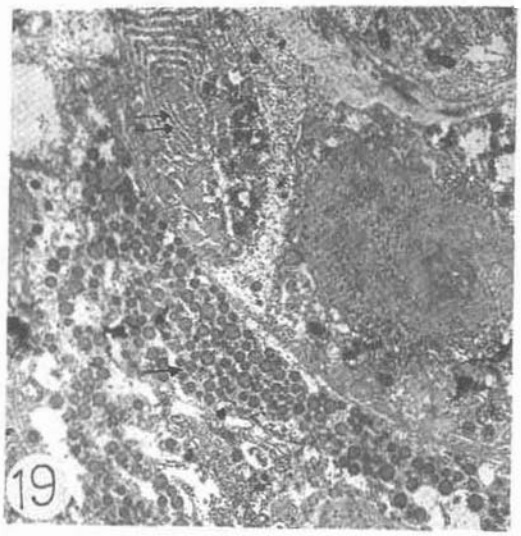


Fig. (19) : An electronmicrograph in the pars distalis of group IV animals showing, a mammotroph with dark irregular nucleus. The cytoplasm contains small amount of granules of variable size and electron density (↑). Note the presence of the soma of another mammotroph containing extensive cisternae of rER with whorly organization (↑↑).

(x 4000)

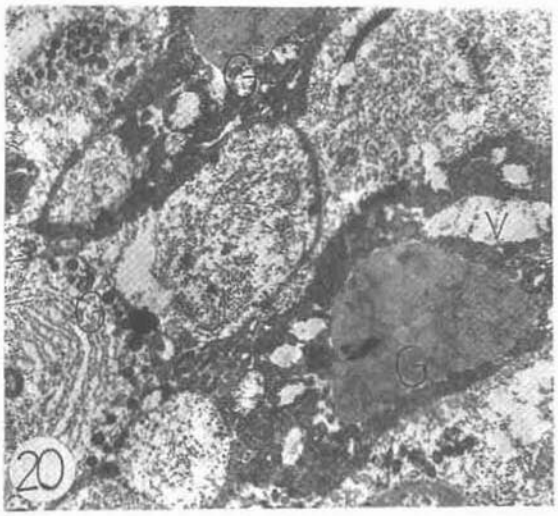


Fig. (20) : An electronmicrograph in the pars distalis of group IV animals showing, parts of 3 gonadotrophs (G) scattered among other types of cells with electron lucent cytoplasm and rerified karyoplasm. The cytoplasm of the gonadotrophs has aggregated dense secretory granules, large vacuoles (V) and highly destructed vaculated mitochondria.

(x 4000)

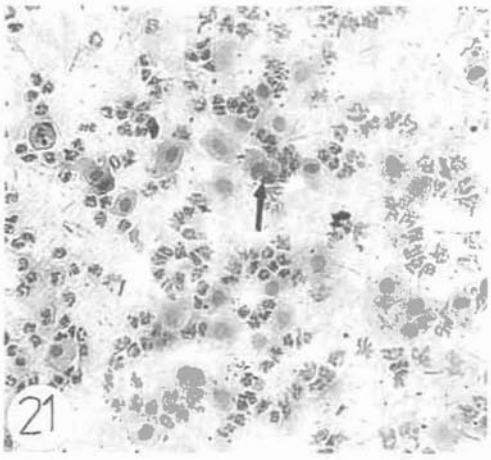


Fig. (21) : A vaginal smear stained with shorr's stain showing a diestrus stage of estrus cycle. Note the presence of a very large number of polymorphs (↑) and some scattered nucleated epithelial cells.

(Shorr's stain; x 200)

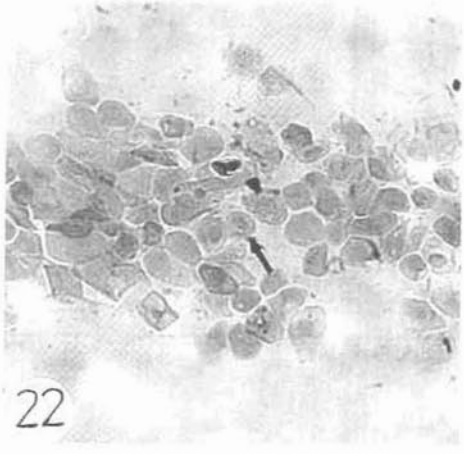


Fig. (22) : A vaginal smear stained with shorr's stain showing, a proestrus stage of estrus cycle. It is characterized by the predominance of nucleated epithelial cells (↑) and the striking decrease in the number of leukocytes.

(Shorr's stain; x 200)

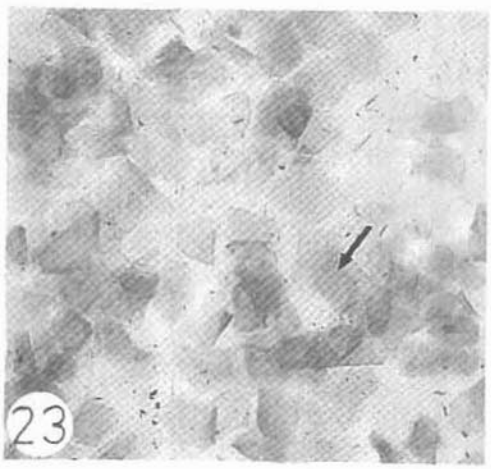


Fig. (23) : A vaginal smear stained with shorr's stain showing an estrus stage of the cycle. Note the cornified polygonal yellow anucleated cells.

(Shorr's stain; x 200)

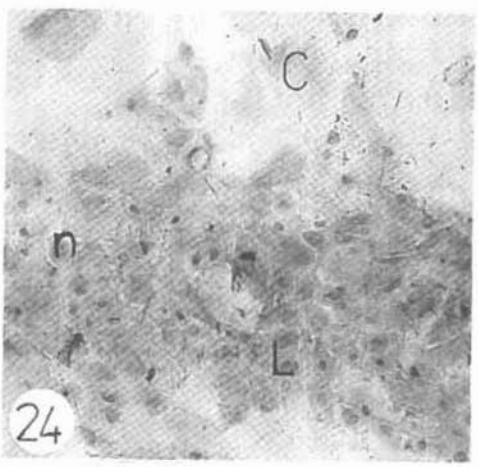


Fig. (24) : A vaginal smear stained with shorr's stain showing, a metestrus stage of the cycle. Note that the three types of cells : cornified cells (C), leukocytes (L) and nucleated epithelial cells (n) are present.

(Shorr's stain; x 200)

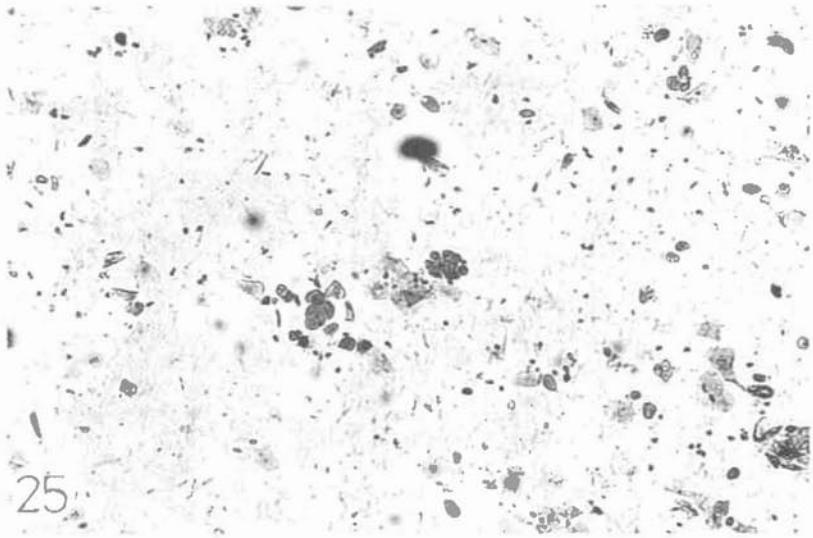


Fig. (25) : A vaginal smear of lactating rat stained with shorr's stain showing, a few number of nucleated epithelial cells (lactational anestrus).

(Shorr's stain; x 100)

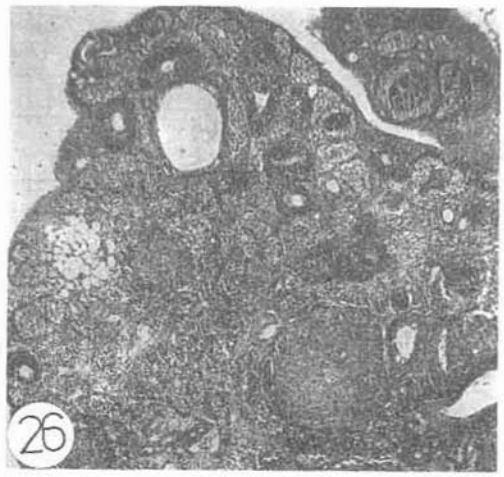


Fig. (26) : A paraffin section in the control ovary at diestrus stage showing, corpus luteum corpus albicans, multiple small follicles in the cortex.

(Hx. & E.; x 40)

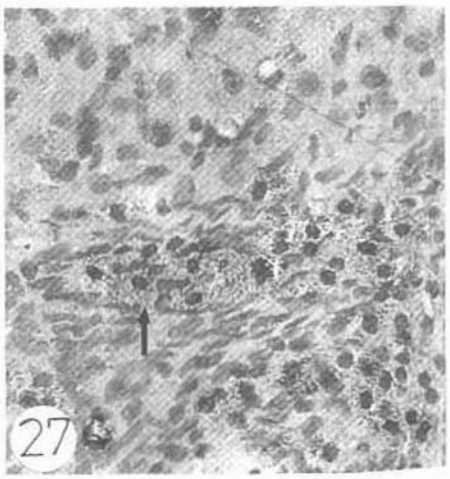


Fig. (27) : Immunohistochemical technique for demonestration of prolactin receptors in the diestrus stroma cells (↑). Note the moderately positive staining in the cytoplasm of the majority of cells.

(x 400)

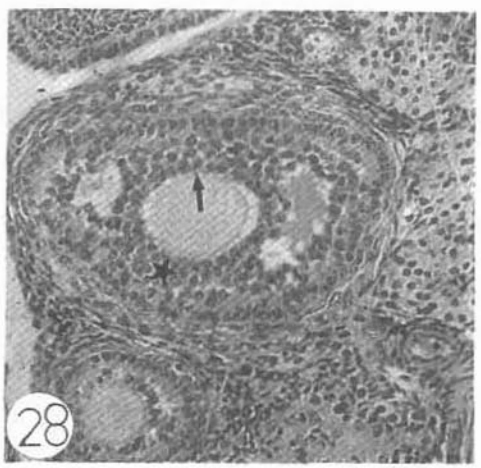


Fig. (28) : A paraffin section in the control ovary at proestrus stage showing growing follicles. The oocyte is surrounded by corona radiata cells (↑). There are multiple layers of granulosa cells with rounded vesicular nuclei (*). Outside basement membrane, there are two or three layers of spindle shaped theca folliculi cells

(Hx. & E.; x 200)

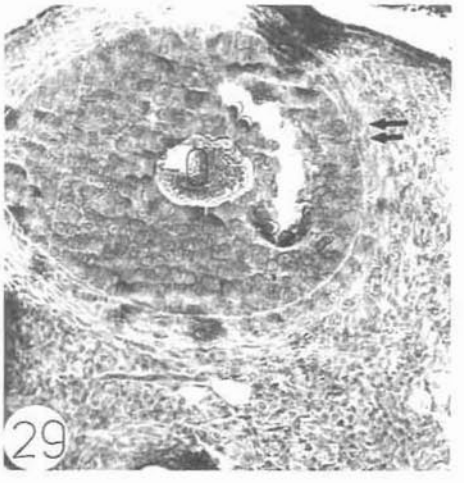


Fig. (29) : An immunohistochemical technique for demonstration of prolactin receptors showing, an intense specific staining of the oocytes (O). An intense specific staining of cytoplasm of corona radiata cells and granulosa cells are also observed. There is weak positive staining of the theca cells (↑↑).

(x 200)

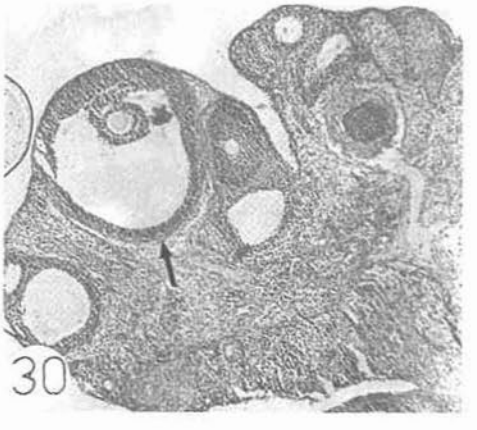


Fig. (30) : A paraffin section in the control ovary at estrus stage showing a mature graffian follicle (↑) and a group of growing follicles.

(Hx. & E.; x 40)

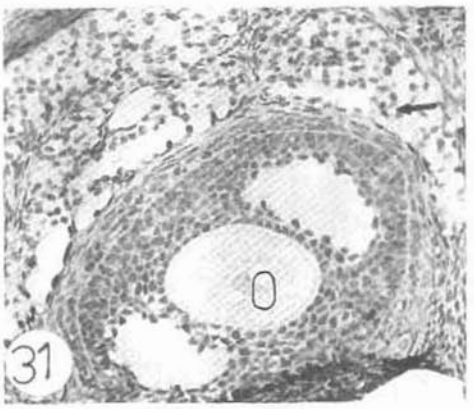


Fig. (31) : An immunohistochemical technique in the estrus ovary showing, a growing follicle. Note the negatively stained oocyte (0), granulosa cells and theca cells. The surrounding stroma cells show weak positive reaction (↑).

(x 200)

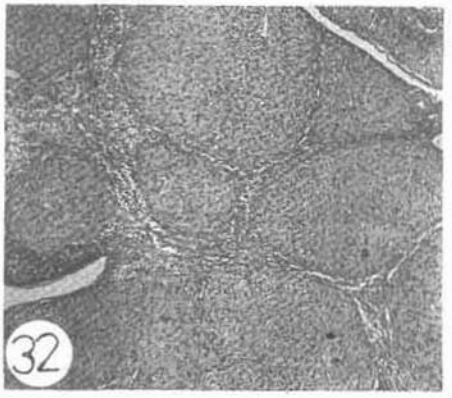


Fig. (32) : A paraffin section in the control ovary at metestrus stage showing, a large number of corpora lutea occupying almost all the ovarian stroma.

(Hx. & E.; x 40)

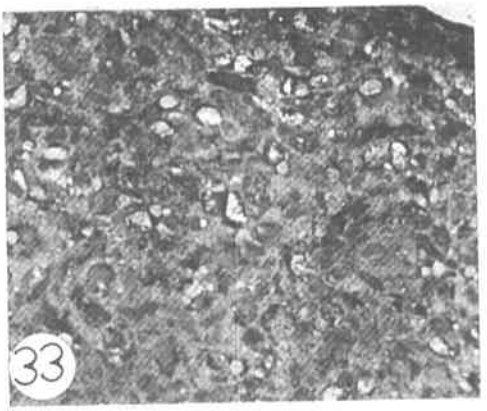


Fig. (33) : An immunohistochemical technique in the metestrus ovary for demonstration of prolactin receptor showing a positive reaction of the cytoplasm in the luteal cells specially concentrated around blood capillaries.
(x 400)

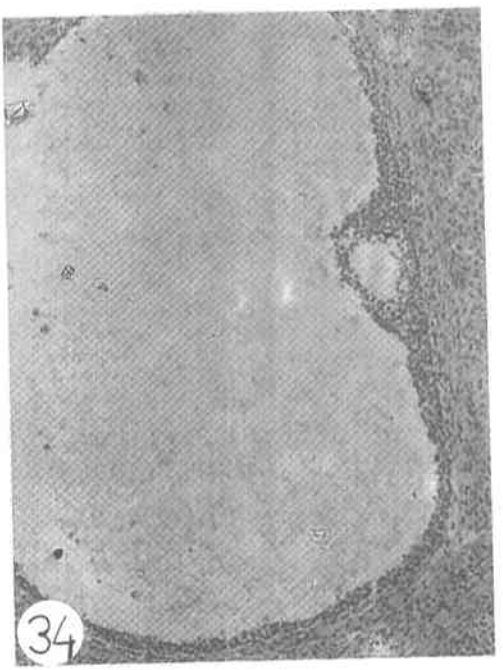


Fig. (34) : A photomicrograph of a paraffin section in the ovary of group II animals showing, a very large cystic follicle with a thin rim of remaining small sized dense granulosa cells. Note that the cumulus oophorus cells surrounding the resorbed oocyte are small sized and dense.
(Hx. & E.; x 100)

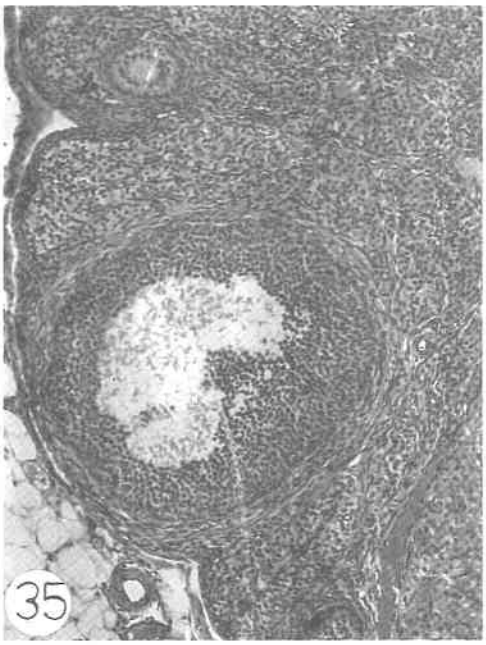


Fig. (35) : A photomicrograph of a paraffin section in the ovary of group II animals showing, a large atretic follicle with no apparent oocyte. The granulosa cells are small and dense. Note the few exfoliating cells in the lumen of the follicle.

(Hx. & E.; x 100)

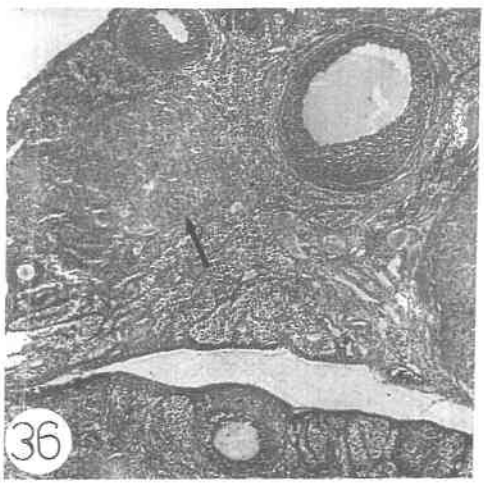


Fig. (36) : A photomicrograph of a paraffin section in the ovary of group III animals showing, multiple cystic follicles of variable sizes in the ovarian stroma. Note the presence of small follicles and degenerated corpus luteum.

(IIx. & E.; x 40)

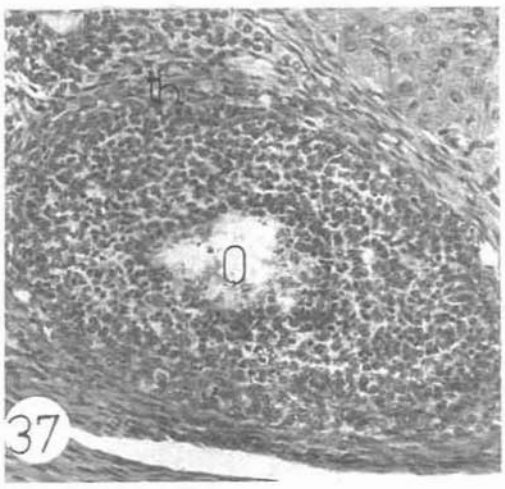


Fig. (37) : A photomicrograph of a paraffin section in the ovary of group III animals showing, secondary follicle with disintegrated oocyte (0) and zona pellucida. Theca folliculi are surrounding the follicle (th).

(Hx. & E.; x 200)

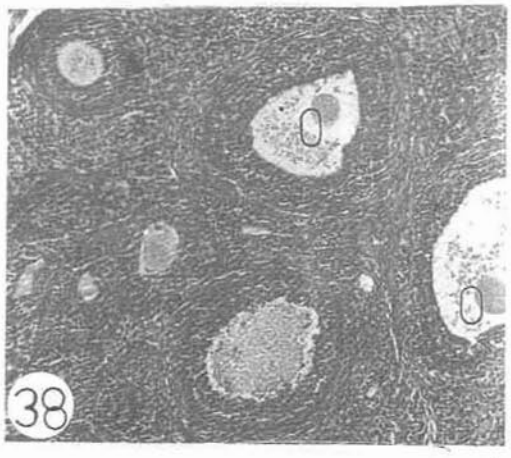


Fig. (38) : A photomicrograph of a paraffin section in the ovary of group IV animals showing, multiple cystic follicles with detached ova (0) inside the lumen of two follicles.

(Hx. & E.; x 40)

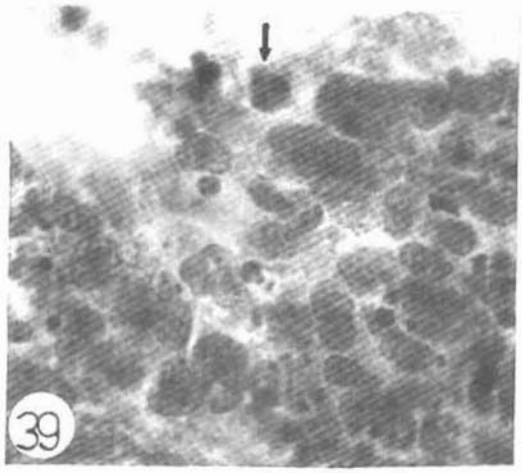


Fig. (39) : A photomicrograph of a paraffin section in the ovary of group IV animals showing a part of follicle with multiple apoptotic bodies. Each dense body surrounded by variable amount of pale cytoplasm.

(Hx. & E.; x 1000)

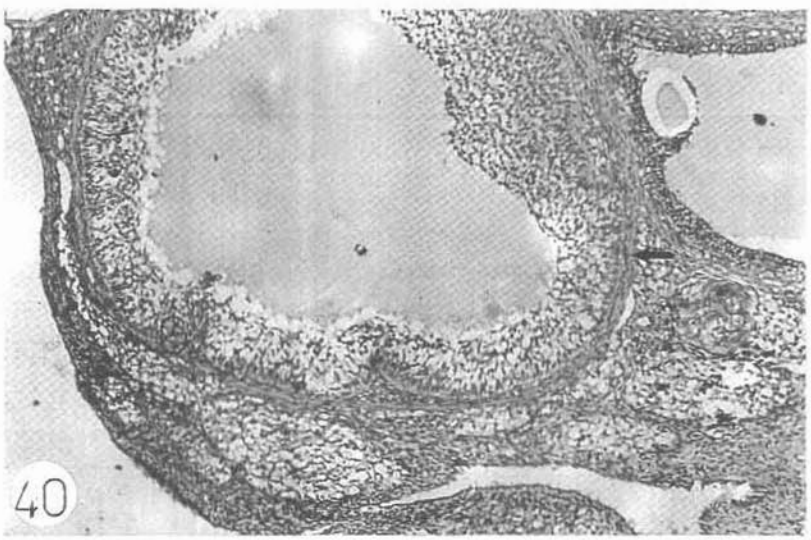


Fig. (40) : Immunocytochemical technique for demonstration of prolactin receptors in the treated ovary (Group IV) showing; weak positive staining of the granulosa cells (G) and negatively stained theca cells (↑).

(x 100)

DISCUSSION

In the present work, the histological changes of experimental hyperprolactinemia induced by administration of sulpiride to intact cycling adult female rats have been investigated. The arcuate nucleus, gonadotrophs and mammotrophs of pars distalis as well as the ovary have been investigated after two and four weeks of treatment. The results were compared with those of lactating and control (non-lactating) rats.

In lactating animals, some neurons of arcuate nucleus showed hyperactivity in the form of invaginated nuclear envelope, organelles rich cytoplasm, with an increase in the immunoreaction for PRL receptors indicating sensitivity of these neurons to the changes in prolactin level. PRL receptor immunostaining in hypothalamic nuclei was previously investigated (**Pi & Grattan, 1998**). The highest number of neurons expressing PRL-R was observed in the arcuate, paraventricular and supraoptic nuclei as these areas play more critical role during lactation (**Pi & Voogt, 2000**). The latter authors reported that increased expression of PRL receptors during lactation is caused by either suckling-induced hyperprolactinemia and / or suckling stimulus itself (**Pi & Voogt, 2001**).

As regard the pars distalis of lactating animal there were manifestations of hyperactivity in the form of predominance of mammotrophs with increase in the amount of secretory granules and some organelles specially rER which were arranged in parallel raws. Gonadotrophs revealed an increase in the amount of secretory granules in immunocytochemical technique. Ultrathin sections showed few dilated strands of rER indicating accumulated FSH and LH inside both the cytoplasm and rER cisternae. These results are in agreement with those reported by **Ciniti et al. (1985)**, who mentioned that in rats with suckling pups LH concentration in pituitary rises, while the plasma LH remains low until the 16th day postpartum. FSH also rises in the pituitary with stable plasma level. This indicates intracytoplasmic hormone storage.

In the ovary of lactating rats, the most characteristic feature was the presence of large cystic follicles. No mature follicles were observed. This may be explained by low serum LH caused by suckling-induced high prolactin level (**Smith & Neill, 1977**) or by the suppressive effect of luteal progesterone that increased in hyperprolactinemic rats (**De-Greef et al., 1995**).

Induction of hyperprolactinemia with sulpiride for two weeks showed signs of activation in the neurons of arcuate nucleus. Besides, nematosmes which are

stigmoid or nucleolus-like bodies were frequently detected in this work in the neuronal somata. The process of nematosomes is considered to be a response to alteration in the peripheral level of sex steroid as was reported by **Shinoda et al. (1992, 1993)**. A marked increase in androgens has been previously reported after the administration of sulpiride (**Ruiz et al., 1984**). Similar activational changes in arcuate neurons have been previously described following administration of progesterone and estrogen (**Vic. et al., 1980**). Therefore, the induced activation changes observed in the present work may result from the influence of estrogen that has possibly been elaborated from aromatization of increased androgens.

In the pars distalis, the activational changes observed in mammotrophs, in the present work, were in the form of large Golgi area, well developed rER as well as dissociated nuclear chromatin. These findings indicate that these cells are typical cells for synthesis and secretion of hormones. Similar activational changes in mammotrophs were previously observed by **Oliveira et al. (1999)**. The usage of many antidopaminergic agents significantly increased the percentage of lactotrophs. Therefore, it is suggested that sulpiride may stimulate prolactin synthesis and secretion by stimulation of lactotroph proliferation by blocking dopaminergic receptors (**Lepola et al., 1989**).

On the other hand, gondotrophs showed accumulation of large amount of secretory granules that were detected both immunocytochemically and ultrastructurally. This indicates impaired release of gonadotropin hormone, despite the increased synthetic activity. These results support the findings that conspicuous increase in prolactin secretion is normally associated with pronounced reduction in gonadotropin secretion, that characterizes stages of infertility. However, the mechanism by which prolactin inhibits gonadotropin release needs further investigations. **Koike et al. (1991)** suggested that excess prolactin decreased the concentration of Gn RH in the rat hypothalamus and thus impaired gonadotropin release from the pituitary gland. On the other hand, **Tortonese et al. (1998)** suggested that prolactin may by involved in the regulation of gonadotropin secretion through a paracrine mechanism within the pituitary gland.

In the ovary, the induced hyperprolactinemia resulted in manifestations of degeneration in the mature graffian follicles. This is in agreement with the previous reports of **Lin et al. (1988)**, who detected failure of follicular rupture and ovulatory dysfunction following sulpiride administration to rabbits. This was explained by inhibition of collagenolytic enzymes activity by sulpiride induced hyperprolactinemia.

Thus, hypersecretion of prolactin may have a direct effect on the ovary by inhibiting follicular rupture induced by human chorionic gonadotrophin.

With longer periods of treatment (4 weeks), there was a progressive increase in the neuronal contents of lipofuscin bodies and peroxisomes. The marked increase in lipofuscin content could be related to the rapid turn over of mitochondria which is the most sensitive organelle to the deleterious effect of hormones especially progesterone as reported by **Grimbert et al. (1995)**. As peroxisomes are normally absent or few in adult rat brain, its increase could be correlated with the mitochondrial affection **(Holtzman, 1982)**.

Another explanation for the increase in peroxisomes is the fact that peroxisomal enzymes utilize substrates that are resistant to mitochondrial oxidation because of the presence of NADP linked isocitrate dehydrogenase, lactate dehydrogenase as an important constituents of peroxisomes **(Dabholker, 1988)**. Several neurons showed highly electron dense nuclei and disorganized organelles. These changes most probably represent degeneration of dark neurons.

In the pars distalis, mammotrophs showed marked regression in the signs of activation which involved both the nucleus and cytoplasm. These changes were considered to be degenerative signs in the rat pituitary cells as previously reported by **Perez & Lawzewitsch (1984)**. Chronic stimulation of mammotrophs might lead to cellular exhausion.

In the ovary, multiple degenerative changes were detected in the follicles including both the ova and the granulosa cells with the presence of multiple apoptotic bodies. An increase in androgen level is known as one of the apoptotic factors in addition to reduction in the gonadotropins which are important factor for the survival of ovarian follicles as was observed by **Hsueh et al. (1996)**.

Immunocytochemical reaction for detection of prolactin receptors in treated rats revealed weakly positive reaction in granulosa cells and negative reaction in theca cells. **Kotok et al. (2000)** investigated the effect of hyper and hypoprolactinemia on the expression of prolactin receptors in various cell types of rat ovaries. They reported that prolactin administration leads to proportional growth of specific immunoreactivity in all cell layers of the granulosa. The administration of bromocryptin, an inhibitor of prolactin secretion, resulted in a decrease in the intensity of specific staining of all cell layers of the granulosa. This variability may be attributed to variabilities in the total binding sites in the ovary under different physiological states.

In conclusion, sulpiride induced hyperprolactinemia produces adverse effects on the arcuate neurons. In the pituitary, it produces activational changes in

gonadotrophs and mammotrophs with occasional degeneration in gonadotrophs followed by induction of degenerative changes in both types of cells. In the ovary, it impairs ovulation and induces degenerative changes in ovarian follicles and corpora lutea.

The action of sulpiride is both centrally, inhibiting gonadotropin release from the pituitary as well as peripherally on the ovary leading to suppression of ovulation. Both mechanisms may be involved. Further investigations as detection of serum level of prolactin, estrogen, progesterone as well as FSH and LH should be done to assure the actual mechanism of action of sulpiride.

SUMMARY

In this work, the effects of hyperprolactinemia induced by sulpiride injection on the structure of arcuate nucleus of hypothalamus, mammotrophs and gonadotrophs of pars distalis and the ovary of adult cyclic female rats were investigated. A total number of 80 rats were used in this study. They are divided into four groups, each of which consisted of 20 animals.

Group I served as control. Group II was lactating animals representing a physiological cause of hyperprolactinemia and two experimental groups. Groups III and IV received intraperitonial injection of sulpiride twice daily in a dose of 100 mg / kg for two and four weeks respectively. Specimens from the arcuate nucleus, pituitary gland and ovary were taken and processed for light and electron microscopic examinations.

Sulpiride induced hyperprolactinemia caused variable structural changes in the arcuate nucleus, pars distalis and the ovary.

At the hypothalamic level, the most characteristic changes observed in the arcuate nucleus of treated animals were those indicative of increased activation in the neurons (irregularity in the nuclear envelope prominent nucleolus and dispersed chromatin).

Dilated RER and Golgi cisternae, multiple lysosome-like dense bodies as well as lipofuscin bodies were detected in the cytoplasm of light neurons. Longer duration of treatment led to subsequent exhausion and appearance of degenerative signs in most of the neurons.

These results were enforced by the usage of immunohistochemical technique for demonstration of prolactin receptors. Their reaction became weak in the treated animals in comparison with that found in the control and lactating animals.

At the level of pituitary, gonadotrophs and mammotrophs became activated in lactating animals and in treated animals for short duration. Then, the secretory granules accumulated in the activated gonadotrophs confirmed by immunohistochemistry, unlike activated mammotrophs which had a small amount of secretory granules. Prolonged duration of treatment led to appearance of degenerative signs in both types of cells.

As regards the ovary, in lactating animals, large unruptured cystic follicles were present. In treated animals, the cystic follicles were more frequently encountered than that in lactating animals. The degenerative changes in the follicles became much more apparent with longer periods of treatment. Multiple apoptotic bodies could be observed in the follicles.

By immunocytochemistry, there was variation in the level of prolactin receptor in the ovary. A reduction in the level of prolactin receptors detected in treated animals is indicative of the direct effect of prolactin on the ovary, in addition to its indirect effect on the hypothalamus and the pituitary.

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الملخص العربى

تاثير زيادة إفراز البرولاكتين المحدث تجريبيا على المحور تحت السريرى النخامى المناسلى فى إناث الجرذان البيض البالغة

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أجرى هذا البحث لدراسة تأثير عقار السلبرايد المستخدم بالحقن على تركيب النواة القوسية فى تحت سرير المخ والخلايا المحفزة للغدة اللبنية والخلايا المحفزة للمناسل والمبيض . وقد استخدم فى هذا البحث ثمانون من إناث الجرذان البيض البالغة حيث قسمت إلى أربع مجموعات تتكون كل منها من عشرين فأرا . المجموعة الأولى ضابطة والثانية مرضعة تمثل زيادة فسيولوجية لهرمون البرولاكتين ومجموعتان تجريبيتان . المجموعة الثالثة والرابعة حقنت بجرعة مرتين يوميا من عقار السلبرايد مقدارها مائة ملليجرام / كيلو جرام فى التجويف البريتونى للبطن لمدة أسبوعين وأربعة أسابيع على التوالى .

وقد أخذت العينات من تحت سرير المخ والجزء القاصى من الغدة النخامية والمبيض وجهزت للفحص بكل من المجهرين الضوئى والألكترونى .

ومن أبرز التغيرات التى ظهرت فى النواة القوسية هو تزايد نشاط الخلايا العصبية مثل تعرجات فى الغشاء المحيط بالنواة مع وضوح شديد للنوية وحدوث انتفاخ فى اجسام جولجى والشبكة الإندوبلازمية الخشنة وأيضا تزايد فى الأجسام المحللة والأجسام الصبغ دهنية والأجسام المحتوية على الإنزيمات فوق المؤكسدة ومع زيادة فترة التجربة لوحظ حدوث تهالك فى معظم الخلايا العصبية .

وباستخدام الدراسة الكيماوية النسيجية المناعية لمستقبلات البرولاكتين وجد أنها تختلف فى المجموعات المختلفة باختلاف نسبة البرولاكتين فى الدم .

أما بالنسبة للخلايا فارزات الهرمون المدر للبن والهرمون المحفز للمناسل فقد وجد زيادة فى نشاط هذين النوعين من الخلايا فى المجموعة الثانية والمجموعتين التجريبيتين وقد تجمعت الحبيبات الإفرازية فى الخلايا فارزات الهرمون المؤثر على المناسل بينما كانت الخلايا فارزات هرمون المدر للبن تحتوى على كميات صغيرة من هذه الحبيبيات ومع زيادة فترة التجربة لوحظ حدوث تهالك فى هذين النوعين من الخلايا وتم تأكيد ذلك بواسطة الدراسة الكيماوية النسيجية المناعية .

أما بالنسبة للمبيض فقد وجد تكيس فى بعض الحويصلات المبيضية وتهالك فى البعض الأخر وقد ظهر التهالك فى عدد محدود نسبيا فى المجموعة المرضعة . أما فى المجموعة التجريبية فقد لوحظ وجود عدد أكبر من الحويصلات المبيضية المتكيسة مع إزدياد التغيرات التهالكية وظهور أجسام دالة على موت الخلايا المبرمج .

وبالدراسة الكيماوية المناعية فى المبيض وجد أن مستقبلات البرولاكتين تتواجد بنسب متفاوتة فى مراحل الدورة المبيضية المختلفة ووجد انخفاض فى مستوى مستقبلات البرولاكتين فى المجموعة التجريبية مما يدل على أن للبرولاكتين تأثيرا مباشرا على المبيضين بالإضافة إلى تأثيره غير المباشر عن طريق النواة القوسية والغدة النخامية .