Structural and Quantitative Changes in the Submandibular Salivary Gland of Ovariectomized Rats

[1]Eman E Abou –Dief, [1]Doha S. Mohamed, [1]Hoda M. Elsayed and [2] Moustafa I. Hassan

[1]Histology Department, Faculty of Medicine, Sohag University, Egypt
[2]Histology Department, Faculty of Medicine, Alazhar University, Egypt
Email: dohasaber@yahoo.com

Abstract: **Background**: Saliva composition shows hormone related changes suggesting that hormones may have a role in the control of salivary gland functions. **Aim of the work:** The aim of this work is to throw light on the effect of female sex hormones on the submandibular salivary gland. **Material and methods**: A total number of 50 adult female Albino rats were used in the present study. Animals were divided into 3 groups as follow: **Group I (GI)**: included 10 animals were used as control. Sham operation was performed for them. **Group II (GII: OVX)**: included 10 animals were bilaterally ovariectomized. **Group III**: included 30 animals: bilaterally ovariectomized and received hormonal replacement therapy 1month after ovariectomy and divided into 3 subgroups; 10 animals each. **Group IIIa:** received estrogen only in a dose of 2.7 microgram/ day orally for one month. **Group IIIb**: received progesterone only in a dose of 0.27 mg orally for one month.**Group IIIc:** received both estrogen and progesterone of the previous doses orally for one month. Sections were obtained and subjected to examination with light microscopy, immunohistochemistry, transmission electron microscopy,morphometric study and statistical analysis: **Results**: Marked light, electron microscopic and morphometric changes were observed as a result of hormonal depletion which improved by hormonal replacement. **Conclusion:** there is an intimate relation between the SMG and the ovarian functions. Monitoring of the latter has been done by several methods like, basal body temperature or by using the cervical mucous but these methods may be misleading.
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Keywords: Submandibular, ovariectomized, progesterone receptors

Introduction

Saliva composition shows hormone related changes suggesting that hormones may have a role in the control of salivary gland functions. Menstrual cycle, pregnancy, and hormone replacement therapy have been shown to affect saliva composition. During the menstrual cycle, specific changes can be observed in saliva composition, e.g. the level of salivary peroxidase **(1)** and secretory Ig A **(2)** in the different phases of the cycle. The inorganic and protein composition of saliva change during the course of pregnancy **(3)**.

Hormonal replacement therapy has been shown to increase saliva flow, has buffering effect and modify protein content of saliva in menopausal women **(4, 5 & 6).** These observations suggest that estrogens may play an important role in oral mucosa and salivary gland physiology. However, the precise mechanism by which estrogens mediate these effects was unclear **(7).**

The effect of estrogens is mediated by estrogen receptors (ERs). Two different types of ERs have been identified, namely ERα (alpha**) (8)** and ERβ (beta) **(9)**. ERα is expressed predominantly in classic estrogen target tissues, such as mammary glands and endometrium. In contrast ERβ is mainly expressed in colonic **(10)** and prostatic epithelium **(9).** ERβ in have been identified in both mucous and serous acinar cells in minor salivary glands, parotid and submandibular glands. The presence of ERs in these tissues suggests a significant clinical importance and predicts a direct role for estrogen in the physiology of oral mucosa and salivary gland function. ERβ may play an important role in the maintenance and function of salivary gland and it is the predominant inducer of progesterone receptors in these tissues **(7)**.

Aim of the work

The aim of this work is to throw light on the effect of female sex hormones on the submandibular salivary gland. To do this we planned to study the histological structure and progesterone receptors of the submandibular gland of female rat in normal conditions, in normal, ovariectomized and in ovariectomized rats supplemented with hormonal therapy. The changes in the latter two experimental conditions will be compared with the former one in a quantitative manner to evaluate the significance of these changes. This would increase the suggestion of the use of saliva as an indicator in determining the normal pattern of female salivary glands under physiological and even some pathological conditions.

Material and methods

Animals

A total number of 50 adult female albino rats were used in the present study. All animals received standard laboratory food and free tap water.

Animals were divided into 3 groups as follow:

Group I (GI): included 10 animals were used as control. Sham operation was performed for them.

Group II (GII: OVX): included 10 animals were bilaterally ovariectomized.

Group III: included 30 animals: bilaterally ovariectomized and received hormonal replacement therapy 1month after ovariectomy and divided into 3 subgroups 10 animals each one.

Group IIIa: received estrogen only in a dose of 2.7 microgram/ day orally for 1 month

Group IIIb: received progesterone only in a dose of 0.27 mg orally for one month

Group IIIc: received both estrogen and progesterone of the previous doses orally for one month.

In Group I and Group II, the animals were sacrificed one month after laparotomy. Group III, animals were sacrificed 24 hours after the last dose of hormonal replacement therapy.

Ovariectomy: It was performed according to **Wayanforth (11)**

Sham Operation:

The abdominal cavities of GI rats were opened but their ovaries were left intact, to be exposed to the same stress of the operation. The same antiseptic precautions and the same way of wound closure were followed as in ovariectomy group.

Preparation of material:

(1)-Estrogen was used as ethinyl estradiol tablets 50 µg produced by Kahira Pharm. & Chem. Ind. Co. Ethinyl estadiol tablets were dissolved as 30 tablets in 100 cc distilled water and each rat received 2.7 µg /day orally by insulin syring for one month.

(2)- Progesterone was used as norethisterone acetate tablets 5 mg produced by CID Company. 30 tablets were dissolved in 100 cc distilled water and each rat received 0.27 mg/day orally by insulin syring for one month.

The dose of estrogen and progesterone replacement therapy was determined according to Paget's equation. The human dose for estrogen and progesterone is 150 µg and 15mg respectively.

Methods:

A- Light microscope

Specimens were fixed in 10% formalin; paraffin embedded and were cut at 5 µm thickness, then stained by:

Immunohistochemistry:

Progesterone receptor (clone SP2), rabbit monoclonal antibody 0.1ml obtained from Medico Company and stored at 4degree in refrigerator was used.

For immunohistochemical staining, precleaned (Superfrost ®)*/Plus-Fisherbrand ®-USA) slides were used. Formalin-fixed paraffin-embedded sections were immunostained using peroxidase-labelled streptavidin-biotin technique to detect progesterone receptors.

Staining procedure*:* was performed according to routine techniques **(12&14).**

Evaluation of immunostaining: For evaluation of progesterone receptor immunoreactivity which appears as brownish black, the entire sections were histologically examined by bright field microscope at low power magnify.

cation (X10) to detect the sites of the antibody positivity. Then higher power Magnification (X40) was used to count number of positive cells. The number of immunoreactive cells was counted in 10 fields(x400) for each animal **(15).**

B-Transmission electron microscopy:

Immediately after sacrificing animals, 10-12 small pieces were obtained from each animal and were fixed in 2.5% glutaraldehyde for 24 hours. Specimens were then washed in 3-4 changes of cacodylate buffer (pH 7.2) for 20 minutes in every change. Specimens were post fixed in cold 1% osmium tetroxide for 2 hours. They were washed in 4 changes of cacodylate buffer for 20 minutes each. Dehydration was done by using ascending grades of alcohol (30, 50, 70, 90, and absolute alcohol) 2 hours for each. Clearing was done in propylene oxide then they were embedded in Epon 812 using gelatin capsule. These samples were kept in incubator at 35 degree for one day, then at 45 degree for another day and at 60 degree for 3 days **(16)**.

Semithin sections (0.5-1um) were prepared by glass knives using LKB ultramicrotome. Sections were stained by Toluidin blue, examined by light microscopy and photographed.

Ultrathin sections (500-800Å) from selected areas of trimmed blocks were made by diamond knives and collected on copper grids. The ultrathin sections were then contrasted in urinyl acetate for 10 minutes and lead citrate for 5 minutes. They were examined and some areas were photographed by electron microscope (Jeol JEM 1010) in electron microscopic unit, Sohag University.

C- Morphometric study and statistical analysis:

Morphometric study was done using image analyzer program at Faculty of Medicine, Al-Azhar University. The measurements included:

(1) The surface area of acini.

(2) The perimeter of acini.

(3) The number of progesterone receptor positive cells.

Data were tabulated and statistically analyzed using SPSS program, student-t was applied to clarify the significant changes in the different groups.

Results

Structural changes in the semithin sections:

Examination of Semithin sections in the control group (Fig.1) revealed that, acinar cells were pyramidal in shape with distinct cell boundaries. They had basal rounded vesicular nuclei. The cytoplasm appeared pale except a thin basal area which was deeply stained.

Numerous capillaries were observed surrounding the acini and ducts. The granular convoluted tubular cells were moderately stained and contained deeply stained granules. Few of them were lightly stained. The striated duct cells were moderately stained.

Semithin stained sections of the ovariectomized group (Fig.2) revealed shrinkage and distortion in the shape of acini. Marked Pyknosis of nuclei and vacuolation of acinar cell cytoplasm was observed.

An increase in the number of cytoplasmic granules of some granular convoluted tubular cells was also observed.

Semithin sections examination of estrogen treated group (Fig.3) revealed less spacing of acini. Some cells were appeared moderately stained with vesicular nuclei; other cells still had cytoplasmic vacuolation but less than ovariectomized group. The granular convoluted tubules had granular cells and non granular cells. The granular contents were less than the ovariectomized group but more than the control one. Dilatation and congestion of blood vessels was observed.

Examination of Semithin sections of progesterone treated group (Fig.4) revealed that, some acinar cells appeared with vesicular nuclei, while others had pyknotic nuclei and vacuolated cytoplasm. The duct system was observed with cells of the granular tubules filled with deeply stained granules.

Semithin sections examination of combined estrogen and progesterone group (Fig. 5) revealed that, some acinar cells had vesicular nuclei. There were less cytoplasmic vacuoles than the ovariectomized group. The granular convoluted tubules were prominent with an increase in the granular contents of its cells.

Ultrastructural study

Electron microscopic examination of the SMG of the control group (Figs.6&7) revealed that acinar cells had basal rounded euchromatic nuclei and apical electron dense secretory granules. Numerous RER cisternae were observed in the basal part of cells.

The striated duct cells **(Fig.8)** were observed to have central rounded euchromatic nuclei and apical short microvilli. Numerous elongated mitochondria were also observed in the basal part of cells.

Electron microscopic examination of the SMG of the ovariectomized group (Figs.9) revealed that acinar cells had irregular pyknotic heterochromatic nuclei and numerous cytoplasmic vacuoles. Some of these vacuoles had electron dense core. Few RER cisternae were also observed.

The striated duct cells **(Fig.10)** were observed to have central rounded euchromatic nuclei and basal infoldings. Some vacuoles were observed in the basal part of cells.

Electron microscopic examination of the SMG of estrogen treated group (Figs.11) revealed that, acinar cells had rounded basal euchromatic nuclei and numerous RER cisternae. Numerous electron dense granules in the apical part of cells were also observed.

The striated duct cells **(Fig.12)** had central rounded euchromatic nuclei, basal infoldings and numerous basal elongated mitochondria.

Electron microscopic examination of the SMG of progesterone treated group (Figs.13) revealed that, the acinar cells had rounded basal euchromatic nuclei and numerous electron dense apical granules. Numerous RER cisternae were also noticed. Some binucleated cells were observed.

The striated duct cells **(Fig.14)** had central rounded euchromattic nclei, basal infoldings and numerous basal elongated mitochondria.

Electron microscopic examination of the SMG of combined treated group (Fig.15) revealed that, the acinar cells had rounded basal euchromatic nuclei and numerous electron dense apical granules. Numerous RER cisternae were also noticed. Some binucleated cells were observed.

The striated duct cells **(Fig.16)** had central rounded euchromatic nuclei, basal infoldings and numerous basal elongated mitochondria.

Immunohistochemical studies of progesterone receptors (PR)

Immunohistochemical study for PR of the control group (Fig.17) revealed a positive reaction in the form of brown to black colour in the nuclei of the intralobular ducts mainly striated duct and also found in the interlobular ducts was not found in the acini or myoepithelial cells.

Immunohistochemical examination of PR of the ovariectomized group (Fig.18) revealed a decrease in the number of positive cells compared to control group. **Immunohistochemical examination of PR of estrogen treated group (Fig.19)** revealed an increase in the number of positive cells compared to previous group. **Immunohistochemical examination of PR of progesterone treated group (Fig.20)** revealed an increase in the number of positive cells compared to the ovariectomized group.

Immunohistochemical examination of PR of combined treated group (Fig.21) revealed an increase in the number of positive cells compared to the ovariectomized one.

Morphometric study and statistical analysis:

.1- Surface area of acini (Table 1& Fig.):

The surface area of acini showed that, it was (153.05±5.40) versus (451.3±28.56) (mean±SE) for the ovariectomized and the control groups respectively.

The surface area of acini was found to be (174.75±4.94) versus (153.05±5.40) (mean±SE) for estrogen treated and ovaoriectmized groups respectively. The surface area of acini was found to be (186.85±6.58) versus (153.05±5.40) (mean±SE) for progesterone treated and ovariectomized groups respectively. The surface area of acini was found to be (230.85±7.64) versus (153.05±5.40) (mean±SE) for combined treated and ovariectomized groups respectively.

2- Perimeter of acini (Table 2& Fig.22):

The perimeter of acini of the control group was found to be 89.26±3.54 (mean±SE).

The perimeter of acini was (53±1.31) versus (89.26) (mean±SE) for the ovariectomized and the control groups respectively.

The perimeter of acini was found to be (53.11±1.42) versus (53±1.31) (mean±SE) for estrogen treated and the ovariectomized groups respectively.

The perimeter of acini was found to be (61.21±6.92) versus (53±1.31) (mean±SE) for progesterone treated and the ovariectomized groups respectively.

The perimeter of acini was (69.27±6.92) versus (53±1.31) (mean±SE) for the combined treated and the ovariectomized groups respectively.

2-Number of PR in positive cells (Table 2 & Fig.24):

The mean number of progesterone receptor positive cells in the control group was found to be 107.6± 8.98 (mean±SE).

The mean number of PR positive cells was found to be (30.53±3.47) versus (107.6±8.89) (mean±SE) for the ovariectomized and the control groups respectively.

The mean number of PR positive cells was found to be (98.4±6.12) versus (30.53±3.47) (mean±SE) for the estrogen treated and the ovariectomized groups respectively.

The mean number of positive cells was found to be (102.1±6.1) versus (30.53±3.47) (mean±SE) for progesterone treated and the ovariectomized groups respectively.

The mean number of PR positive cells was found to be (99.23±6.1) versus (30.53±3.47) (mean±SE) for the combined treated and the ovariectomized groups respectively.

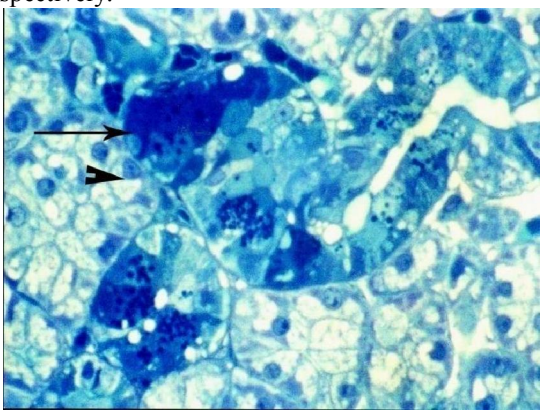


Fig (1): Semithin section of the SMG of (GI) showing acinar cells with vesicular nuclei& moderately stained cytoplasm (arrow head). Note G.C.T. with apical deeply stained granules (arrow). (Toluidine blue X 1000)

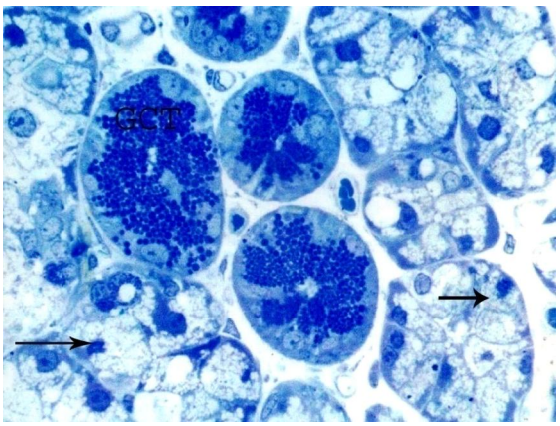


Fig (2): Semithin section of the SMG of (GII) showing pyknosis in some acinar cells nuclei with vacuolated cytoplasm (arrows) and increased granular contents of granular convoluted tubules (G.C.T) compared to (GI). (Toluidine blue X 1000)

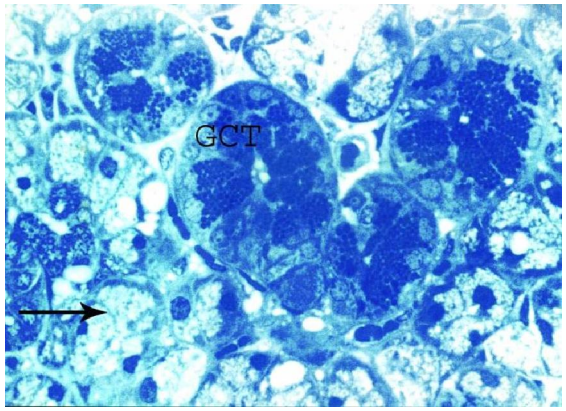


Fig (3) Semithin section of the SMG of (GIIIa) showing some acinar cells and increased granular contents of some granular convoluted tubular cells(G.C.T) compared to (GI). (Toluidine blue X1000)

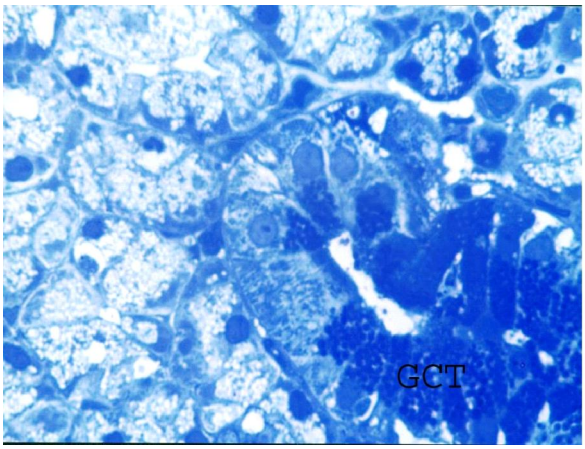


Fig (4) Semithin section of the SMG of (GIIIb) showing prominent granular convoluted tubules (G.C.T) with increased granular contents of their cells compared to (GI). (Toluidine blue X1000)

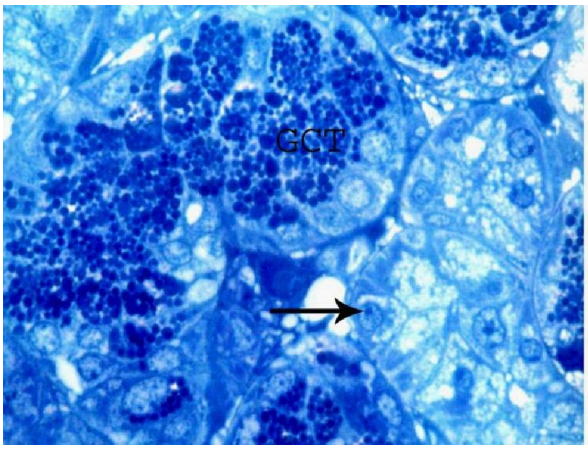


Fig (5): Semithin section of the SMG of (GIIIc) showing vesicular nuclei of acinar cells (arrow). Note,prominent granular convoluted tubules with increased granular contents of their cells(G.C.T). (Toluidine blueX1000)

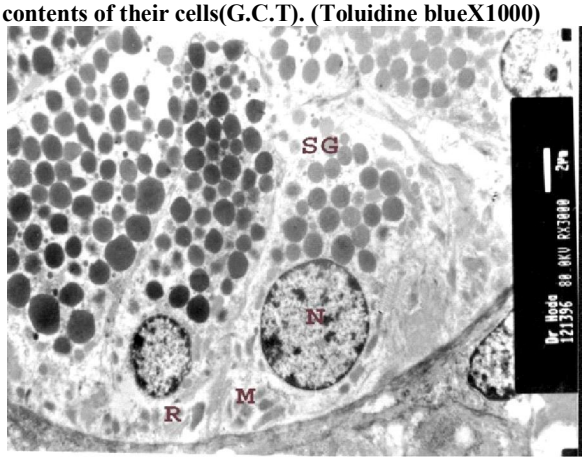


Fig (6): Electron micrograph of an acinus of SMG of (GI) showing serous acinar cells with basal euchromatic nuclei (N) and apical electron dense secretory granules (SG).
(X3000)

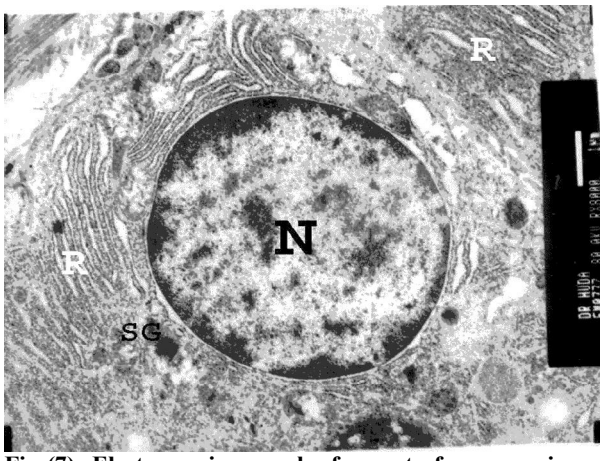


Fig (7): Electron micrograph of a part of serous acinar cell having euchromatic basal rounded nucleus (N), numerous RER cisternae(R) and secretory granules (SG). (X8000)

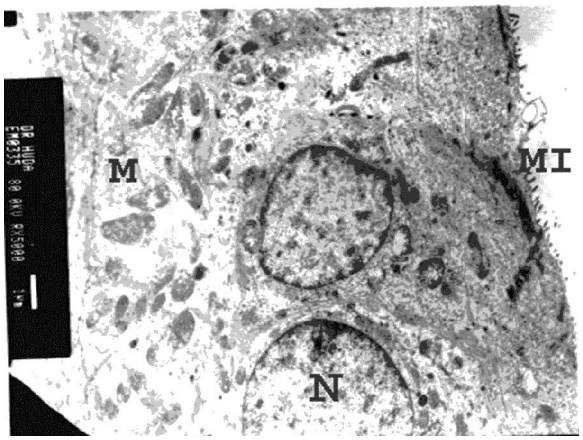


Fig (8): Electron micrograph of adjacent striated duct cells of (GI) showing euchromatic central nucleus (N), numerous mitochondria (M) and apical microvilli(MI). (X5000)

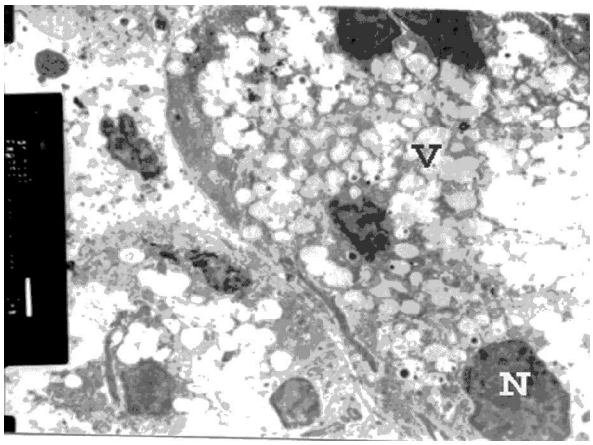


Fig (9): Electron micrograph of the SMG of (GII) showing acinar cells with pyknotic heterochromatic nuclei (N) and cytoplasmic vacuoles (V). (X3000)

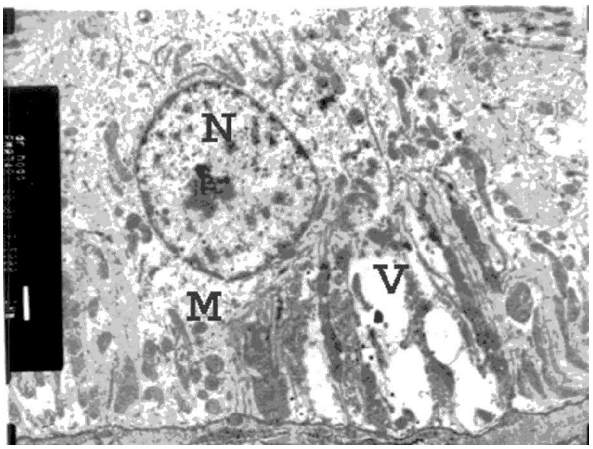


Fig (10): Electron micrograph of the striated duct cells of (GII) showing central euchromatic nucleus (N), basal mitochondria (M) and cytoplasmic vacuoles. (X5000)

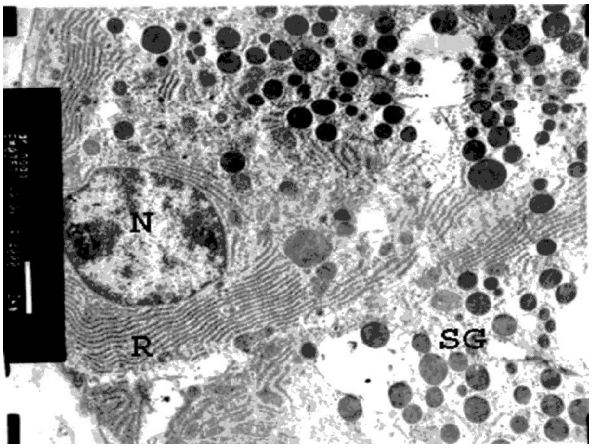


Fig (11): electron micrograph of the SMG of (GIIIa) showing parts of 2 adjacent acinar cells each has rounded basal euchromatic nucleus (N), numerous RER cisternae and numerous apical electrone dense secretory granules (SG). (X3000)

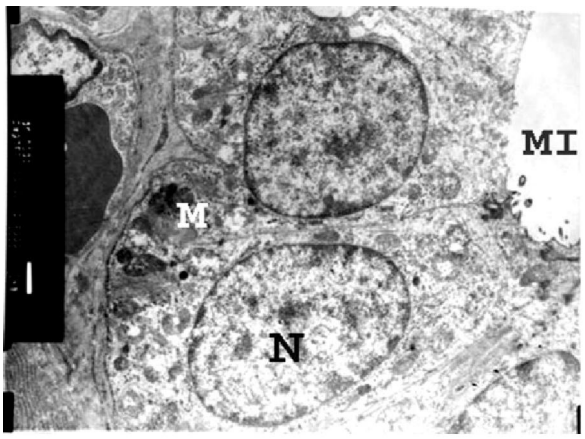


Fig (12): Electron micrograph of the striated duct cells of (GIIIa) showing central euchromatic nucleus (N), basal small mitochondria (M) and apical short microvilli (MI). (X5000)

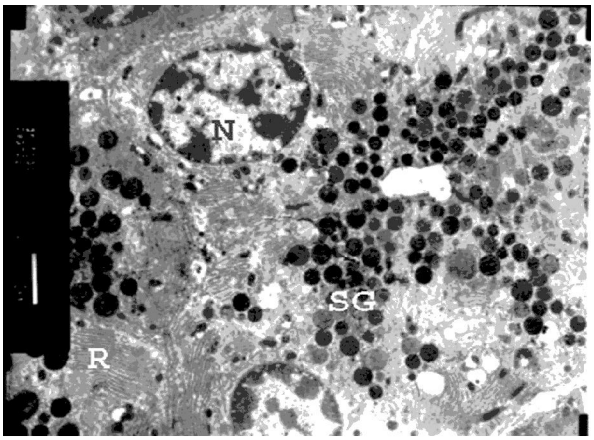


Fig (13): An electron micrograph of an acinus of the SMG of (GIIIb) showing acinar cells, each had rounded euchromatic nucleus (N), apical electron dense secretory granules (SG) (X3000)

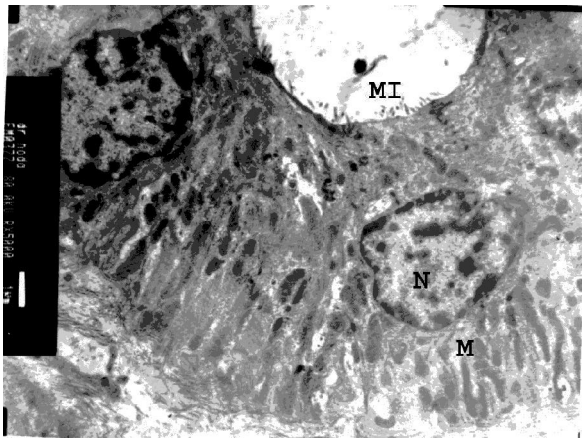


Fig (14) An electron micrograph of a striated duct cells of (GIIIb) showing euchromatic central nucleus (N), basal elongated mitochondria (M) and apical short microvilli (MI). (X5000)

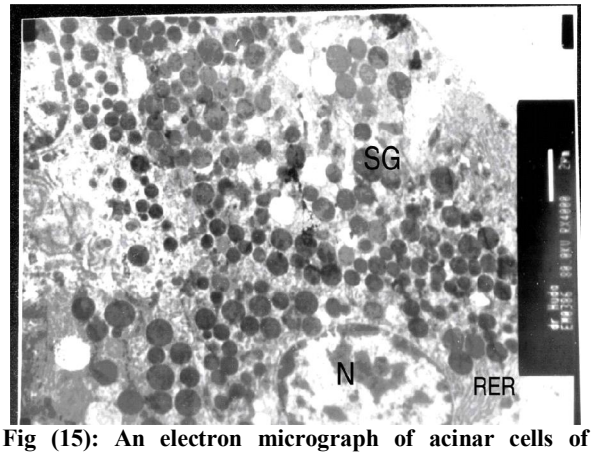


Fig (15): An electron micrograph of acinar cells of (GIIIc) showing rounded euchromatic nucleus and numerous apical electron dense secretory granules. (X4000)

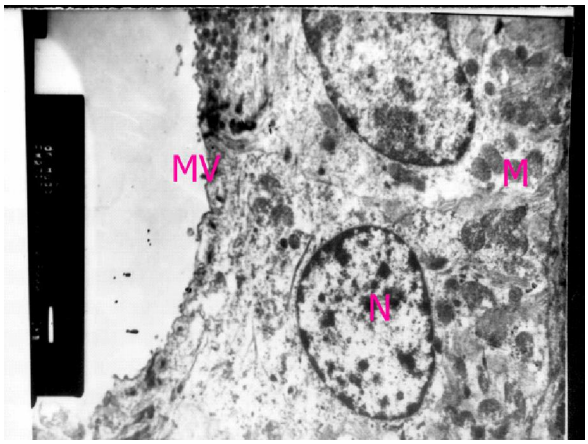


Fig (16): An electron micrograph of a striated duct cells of (GIIIc) showing euchromatic central nucleus (N), basal elongated mitochondria (M) and apical short microvilli (Mv). (X5000)

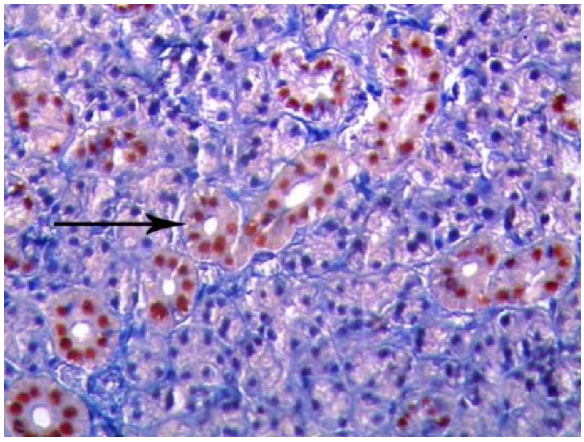


Fig (17): A photomicrograph of the SMG of (GI) showing positive reaction for progesterone receptor in the nuclei of intralobuar ducts mainly striated ducts (arrow). (Immunohistochemistry for PRX400)

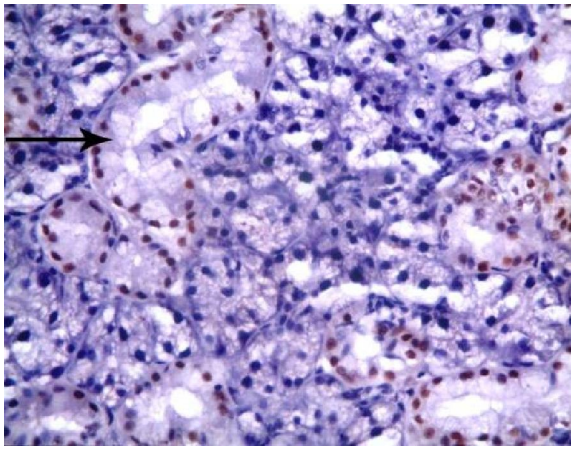


Fig (18): A photomicrograph of the SMG of (GII) showing a decreased number of progesterone receptor positive cells (arrow) (immunohistochemistry for PRx400)

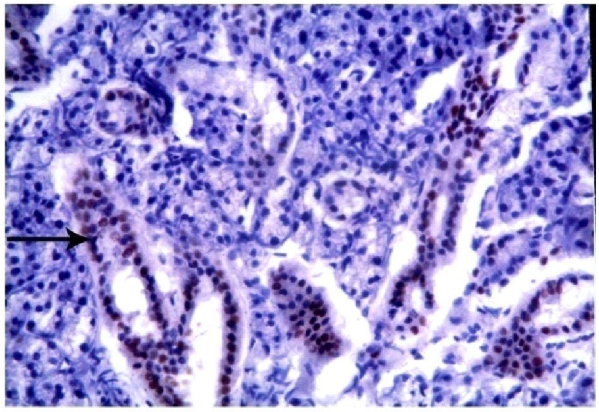


Fig (19): A photomicrograph of the SMG of (GIIIa) showing an increased number of progesterone receptor positive cells (arrow) compared to (GII). (Immunohistochemistry for PR (X400)

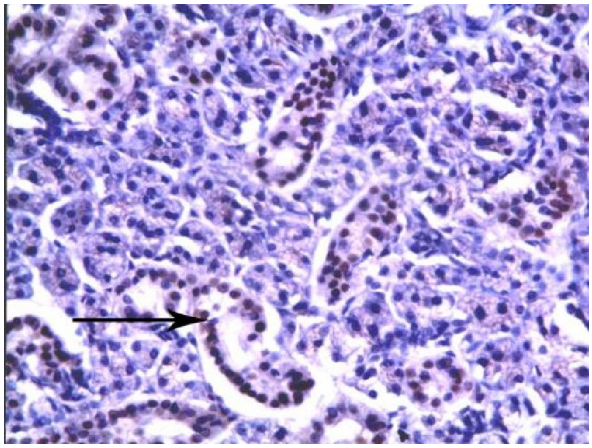


Fig (20): A photomicrograph of the SMG of (GIIIb) showing increased number of progesterone receptor positive cells compared to (GII) (Immunohistochemistry for PRX400)

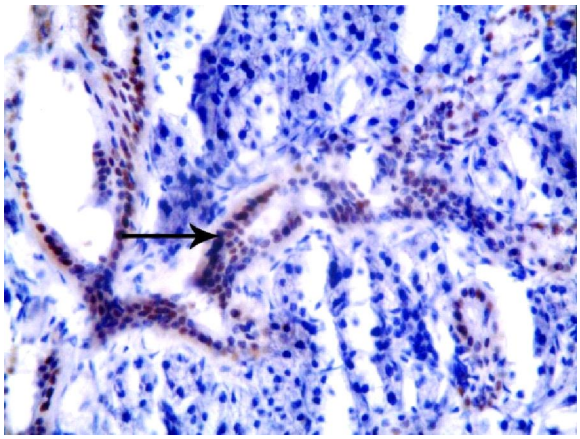
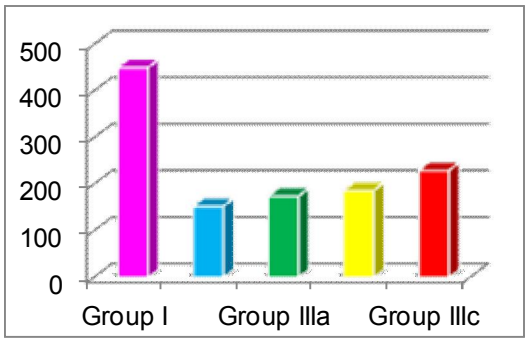


Fig (21): A photomicrograph of the SMG of (GIIIc) showing increased number of progesterone receptor positive cells (arrow) compared to (GII) (immunohistochemistry for PRX400)

Table (1): surface area of acini

	GI	GII	GIIIa	GIIIb	GIIIc
MEAN	451.3	153.05	174.75	186.85	230
SD	127.7	24.17	22.12	29.47	34.17
SE	28.56	5.40	4.94	6.58	7.64
P value1		<0.0001	<0.0001	<0.0001	<0.0001
SIG.		***	***	***	***
P value2	<0.0001		0.005	0.0003	<0.000
SIG.	***		***	***	***
P value3	<0.0001	<0.000	<0.0001	0.0001	
SIG.	***	***	***	***	

SD: standard deviation
SE: standard error
P value1: compared to Group I
Pvalue2: compared to GII
Pvalue3: compared to GIIIc
SIG: significance
*** Highly significant P value<0.0001

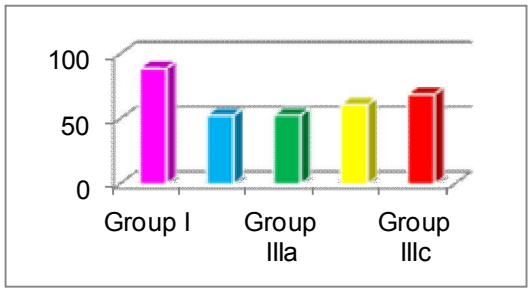


(Fig.22) Changes in the mean surface area of acini in the different groups of the study

Table (2): perimeter of acini:

	GI	GII	GIIIa	GIIIb	GIIIc
MEAN	89.26	53	53.11	61.21	69.27
SD	15.83	5.86	6.36	8.81	7.45
SE	3.54	1.31	1.42	1.97	1.67
P value1		<0.0001	<0.0001	<0.0001	<0.0001
SIG.		***	***	***	***
P value2	<0.0001		0.71	0.004	<0.0001
SIG.	***		NS	***	***
P value3	<0.0001	<0.0001	<0.0001	0.003	
SIG.	***	***	***	***	

SD:standard deviation
SE: standard error
P value1: compared to Group I
Pvalue2: compared to GII
Pvalue3: compared to GIIIc
SIG: significance
***: highly significant P value<0.001

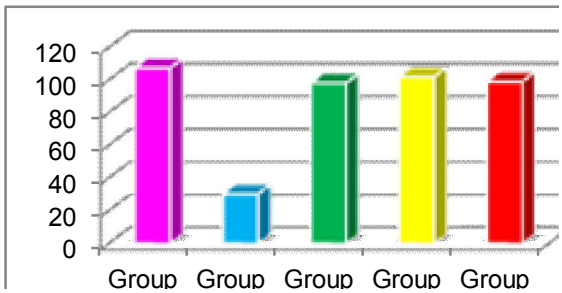


(Fig.23) Changes in **the perimeter of acini in the different groups of the study

Table (5): Number of progesterone receptor positive cells

	Group I	Group II	Group IIIa	Group IIIb	Group IIIc
MEAN	107.6	30.53	98.4	102.1	99.23
SD	39.80	18.98	6.12	42.26	29.92
SE	8.89	3.47	19.36	13.36	5.46
P value1		<0.0001	0.49	0.73	0.40
SIG.		***	NS	NS	NS
P value2	<0.0001		<0.0001	<0.0001	<0.0001
SIG.	***		***	***	***
P value3	0.40	<0.0001	0.94	0.81	
SIG.	NS	***	NS	NS	

SD: standard deviation
SE: standard error
SIG: significance
*** Highly significant P value<0.0001
1-compared to group I
2-compared to group II
3-compared to group IIIc



(Fig.24) Changes in the mean number of progesterone receptors positive cells in the different groups of the study

Discussion

The effect of various hormones on the SMG is a matter of interest by many scientists. It was reported that mammalian SMG is a target tissue for both estradiol and progesterone hormones (15). In this study a focus was applied on the structural and morphometric changes in the SMG of ovariectomized rat and after the hormonal replacement aiming to prove that salivary glands may play a role, in the near future, in the diagnosis of some gynecological and obstetrical problems associated with hormonal imbalance.

Microscopic examination of the control SMG group showed that the gland consisted of stroma and parenchyma. The parenchyma consists of secretory acini and duct system. The acini were found to be of the serous type and surrounded by myoepithelial cells. Ultrastructural examination of the acinar cells in the control group revealed the characters of protein secreting cells in the form of basal rounded euchromatic nuclei and apical electron dense secretory granules with numerous RER cisternae in the basal part of the cells. The principle protein products of acinar cells are the enzymes amylase, lysozyme peroxidase, and deoxyribonuclease **(17)**. It was reported that, there are interspecies and strain differences in the histological and histochemical

structure of the rodents' SMG. They attributed these differences to the feeding habits **(18).**

The duct system included the intercalated duct, granular convoluted tubule (G.C.Ts), striated duct and excretory ducts. The striated duct cells were observed to have basal striations and apical brush border. Ultra structure examination revealed the presence of central rounded euchromatic nuclei,apical short microvilli and numerous elongated mitochondria in the basal part of such cells. The G.C.Ts were observed to have basal rounded nuclei and acidophilic cytoplasm. **The G.C.Ts** were observed to have granular and non-granular cells. The former had rounded basal euchromatic nuclei and apical electron dense granules. They had also basal infoldings and numerous basal elongated mitochondria. Both striated duct and G.C.Ts have the criteria of ion transporting cells. They have a role in modifying the chemical composition of saliva and contain both estrogen and progesterone receptors (7). They are the specific duct segment in rodents and under androgen hormonal control and are the site of secretion of many peptides. These factors are suggested to function as exocrine salivary factors or endocrine factors after reabsorption **(19)**. Although the gland contains a single type of acini it is considered mixed, as the serous secretion produced by the cells of the serous acini is added to the secretion of the cells of G.C.Ts **(20)**.

The SMG of ovariectomized group showed acinar cells with manifestations of both necrosis and apoptosis. Degenerative changes in the form of shrinkage and distortion in the acinar shape with pyknotic or heterochromatic nuclei and cytoplamic vacuolations was demonstrated. Some of these vacuoles had electron dense core. Vacuolation may be explained as dilated RER cisternae or swollen mitochondria with destructed cristae. Cellular vacuolation were noticed previously after ovariectomy in other cells such as aortic endothelial cells (**21)** and in acinar cells of lacrimal gland of rabbits (22)**.** vacuolation may be due to functional loss and disturbed structural integrity of their membranes in the absence of nuclear fragmentation. Necrosis in the acinar areas could be secondary response to loss of trophic paracrine factors produced by interstitial cells eliminated by apoptosis **(23&22).**

These findings confirm that ovariectomy caused changes in the parenchyma similar to those of aged SMG as the same changes were observed in aged rat SMG of by many authors (24,25&26) and in aged human parotid gland (27). It is well known that in normal adult tissue there is a balance between cell renewal and cell death by apoptosis. Aging causes an increase in the rate of apoptosis in acinar cells with insignificant change in the rate of apoptosis of duct cells. Sex hormones could influence age related histological changes induced by apoptosis as previously postulated in ovariectomized rat parotid gland **(13)** and in lacrimal gland (22).

The duct system in ovariectomized group also showed some degenerative changes. Cells of the striated duct showed some vacuolation in the basal parts. The G.C.Ts showed an increase in the granular content of their cells. This means that absence of female sex hormones led to the appearance of the G.C.Ts as its appearance in males as **(28)** described this pattern of the G.C.Ts as androgen phenotype. reported that the area of the G.C.Ts in males was significantly greater than in female, corroborating to the presence of sex dimorphism in this structure **(28)**. It was hypothesized that this sexual dimorphism was due to gland specific difference in gene expression between males and females **(29)**.

The SMG of ovariectomized group and treated with estrogen or progesterone showed improvement of some of the degenerative changes reported in ovariectomized group, and the improvement was maximally manifested in combined treated group. These manifestations included the closely packed acini with vesicular or euchromatic nuclei of acinar cells. Few or no cytoplasmic vacuolations were observed. Examination of the striated duct cells revealed that they were more or less similar to the control group. The G.C.Ts showed an apparent increase in their granular contents compared to the control group but still less than those demonstrated in the ovariectomized group. The group of combined estrogen and progesterone treated ovariectomized rats revealed that the acini and duct system were more or less similar to the control group. The effect of steroid hormones on various tissues has been Previously demonstrated. In a previous research It was found that estrogen administration to ovariectomized rats reduced cellular vacuolation in aortic endothelial cells indicating the role of estrogen in maintaining the normal structure of such cells **(21)**. It was reported that estradiol administration to ovariectomized rats caused a decrease in the number of granules in the G.C.Ts. This controversy with our results may be explained by difference in the type and duration of estrogen administration in such study. The increase in the granular contents of the G.C.Ts may be explained that estrogen and progesterone affects the protein secretion in the G.C.Ts which is the site of NGF and EGF secretion **(30)**. In a previous study it was stated that the levels of these factors increased after progesterone administration to ovariectomized rats**(31).** Previously it was proposed that progesterone had also a modulating effect on the protein component and amino acid composition of the secretory granules of the intralobular duct cells of the rat**(32)**. Furthermore, progesterone was reported to be

necessary for trypsin-like protease activity located in the secretory granules. An additional indicator for the important role of progesterone in regulating the secretory activity of mammalian SMG was given by a study revealing that rodent SMG contain metabolizing enzymes that transform progesterone to ring A-reduced derivative with biological activities different from those of the parent hormone **(33)**.

Analysis of the morphometric measurements indicate that both estrogen and progesterone are necessary for maintaining the normal parenchyma of the SMG in rats. The surface area of the SMG acini significantly decreased in ovariectomized group compared to the control one ((153.05±5.40 versus 451.3±28.56) and it was significantly increased in ovariectomized and treated with estrogen,progesterone and combined treatment (174.75±4.94, 186.85±6.58 and 230.85±7.64 respectively) versus the ovariectomized group. The perimeter of the SMG acini significantly decreased in ovariectomized group compared to the control one (53±1.31 versus 89.26±3.54) and it was significantly increased in ovariectomized and treated with estrogen, progesterone and combined treatment(53.11±1.42, 61.21±6.92 and 69.27±6.92 respectively) versus the ovariectomized group. In accordance with this study, it was reported a significant decrease in the percentage of acini in the SMG of ovariectomized rats **(35)**. In addition, ovariectomy resulted in a significant decrease in the parenchyma to stroma ratio in the SMG of rats which is associated with a significant decrease in the stimulated submandibular salivary flow rate as stated previously that acinar surface area in estrogen treated ovariectomized rabbits (22) and rats**(34)**. It was reported that, progesterone treatment of ovariectomized rats leads to a significant increase in the parenchymatous area of mammary gland**(36)**.

The parenchyma of the SMG in the adult rat is thought to be self-renewing. The presence of estrogen and progesterone receptors in the SMG indicates that both hormones are important in maintaining its parenchyma. The significant decrease in the percentage of acini in the SMG of ovariectomized rats might be attributed to the increase in the rate of apoptosis due to estrogen deficiency and the reduced transcription signals for cell cycle involved with estrogen receptor (13)**.** Combined estrogen and progesterone treatment of ovariectomized rats is known to increase the parenchymatous area of other glands which proved to have both ER and PR such as lacrimal gland **(36)** and mammary gland **(37).** The possible explanation of mentioned changes may be attributed to the fact that both hormones may suppress the atrophic changes through suppression of apoptosis. Estrogen is known to upregulate the expression of antiapoptotic factors and down regulate the expression of proapoptotic factors in some tissues as nervous tissue **(38).** This may refered to the possibility for these hormones to induce altered activity of cell death, signal transduction, receptor, endocrine, enzymatic, and growth factor genes as previously reported in lacrimal glands**(36).**

PR was demonstrated in this study in the nuclei of the intralobular ducts mainly striated duct and also found in the interlobular ducts. The distribution of these receptors in the parenchymal cells is a subject of controverse. Similar finding was observed in the SMG of rat **(39)** and human **(40)** & **(41) but** in rabbits they were localized in the nuclei of the acinar cells which reflect a species difference in the distribution of PR**(15)**.

The mean number of progesterone receptor immunoreactive cells significantly decreased in ovariectomized group compared to the control one (30.53±3.47 versus 107.6± 8.98) **(39)** studied the effect of both ovariectomy and orchidectomy on the number of PR in female and male rats. They reported that ovariectomy resulted in a decrease in the number of PR positive cells while in males, orchidectomy caused an increase in their number when compared to the control rats.

Morphometeric measurements of PR immunoreactive cells revealed that they are increased in estrogen treated ovariectomized rats.It was 98.4±6.12 versus 30.53±3.47 in the ovariectomized group. Similar findings were previously reported in the SMG of rats by **(39).** This can be attributed to up regulation of PR by estrogen. It was reported that**,** down regulation of PR by ovariectomy and its upregulation by estradiol occur in many traditional progesterone target tissues including; uterus **(42),** vagina, fallopian tube **(5)** and mammary gland **(6)** as well as in nontraditional target tissues like; eye **(43),** brain, pituitary gland and urethrab **(44)**.The distribution of ERβ is in the same sites of PR in the ductal cells which are known to have a significant role in modulating the inorganic composition of saliva suggests that ERβ is the predominant inducer of PR **(7)**. The presence of estrogen receptors (ERs) in the SMG has been reported in many studies in rats **(45),** rabbits **(15)** and human **(7).** Estrogen is believed to mediate its effects by binding to the products of two related genes, ER α and ER β, members of the steroid receptor branch of the nuclear receptor superfamily **(46) & (47).**

Progesterone administration to ovariectomized rats caused an increase in the number of PR positive cells compared to ovariectomized group. It was 102.1±6.1 versus 30.53±3.47 in the ovariectomized group. This may be due to the role of progesterone on the expression of PR in the SMG **as** reported that, endogenous PR is located in the form of nuclear

aggregates only in the secretory phase of the menstrual cycle in human, when progesterone levels are high**(48)**. Progesterone has also a role in the activity of other glands such as the lacrimal gland. It induces significant changes in the expression of genes involved in signal transduction and cell communication, metabolism and cell growth and/or maintenance**(36).**

In the present study, combined treatment with estrogen and progesterone after ovariectomy resulted in an apparent increase in the number of PR positive cells compared to the ovariectomized group. It was 99.23±6.1 versus 30.53±3.47 in the ovariectomized group. This ensures the fact that both estradiol and progesterone are regulators of PR expression in the rat SMG**.** Combined treatment with estrogen and progesterone up-regulates the mRNA expression of the PR in the ovary and the uterus of Mongolian gerbils**(49).**

The interaction between the SMG and the reproductive system is a mutual relation both in male and female. Sialoadenectomy markedly reduced the aromatase activity, an enzyme needed for conversion of androgen to estrogen. Several studies support the possibility of an endocrine role of SMG-derived EGF on reproductive organs. It was stated that such factor stimulates the meiotic phase of spermatogenesis in male mouse **(50)** and maintain uterine growth, fertility and mammary gland development in females **(51).** Ablation of SMG in male rats results in atrophy of testicular interstitial tissue (52)**.**

From this study, it was clear that there is an intimate relation between the SMG and the ovarian functions. Monitoring of the latter has been done by several methods like, basal body temperature or by using the cervical mucous but these methods may be misleading. Saliva ferning (arborization) test as a simple and cheap method as a new parameter for evaluation of the ovarian function in different physiological and pathological conditions, saliva may have a role in diagnosing pregnancy and evaluating hormonal status in female.

It is highly recommended and may be considered as a new parameter in evaluating the functions of female hormones. Additional studies essential to be applied on humans especially many medical companies announce for many products that can diagnose pregnancy through saliva.

Correspondence to
Dr Doha Saber Mohammed
Department of Histology, Faculty of Medicine, Sohag University, Sohag, Egypt
e-mail: dohasaber@yahoo.com
Tel: 01007843762
Fax: 0934602963

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العـــربى الملخـص

اللعابيـــــة الغـدة فـي الكميـــة و التركيبيـــــة التغـــيرات المستأصـــــلة الجـرذان فـــي الفكيـــة تحـت المبيضـــــين
محمد هدى*، محمد صـــابر ضـحى *،ضـيف بـوأ السـيد يمـان إ* سـيد
حسـن سـماعيل إ مصـطفى**
مصر.سوهاج معة جا كليـــةالطب هســـتولوجيال قســم *
مصر.الأزهر معة جا بنيـــن طب كليـــة الهســـتولوجى قســم **

تغـــيراتلل تبعـــا اللعـــاب تكـــوين يختلـــف :الخلفيـــــة دور لها يكـون قـد الهرمونـات بـأن يوحـي مما الهرمونيـة من الهدف .اللعابيـــــة الغـدة وظـائف علـى السـيطرة فـي الضـوء تســـليط هو الدراسـه هذه من والهدف :الدراسـه علـى الأنثويـــة الجنســـية الهرمونـات تـــأثير علـى هذه فـي ..الســـفلي الفـك تحـت اللعابيـــة الغـدة البالغـــه الجـرذان انـاث نم 50 عدد اسـتخدام تـم الدراسـة ثـلاث إلـى الحيوانـــات تقســـيم تـم :والأســـاليب المواد شـملت الاولـى المجموعة :التـــالي النحـو علـى مجموعات المجموعة .لهم صـورية عمليـة إجراء تـم الحيوانـــات 10 استئصـــال لهم تـم حيوانـات10 وشـملت :الثانيـــه تـم حيوانـا30 وشـملت الثالثـــه المجموعة المبيضـــين جرعات اعطـائهم مع لهم لمبيـــضا اسئصـــال وتـم شهر لمده الانثويـــه الهورمونـات من تعويضـــيه وتقســـيمها :فرعيـــة مجموعات ثـلاث الـى تقســـيمهم المجموعه مجموعه لكـل حيوانـات 10 بمجموعات 3 إلـى فـي فقـط الاســـتروجين تلقـت :Group IIIa الاولـى لمدة الفـم طـريق عن يـوم / ميكـروجرام 2.7 من جرعة تلقـت :Group IIIb.الثانيـــه عشـهرالمجمو طـريق عن مج 0.27 بجرعـة فقـط البروجســـترون تلقـت :Group IIIc الثالثـــه المجموعه شهر لمده الفـم بالجرعـات والبروجســـتيرون الاســـتروجين من كل الحصـول تـم .شهر لمدة الفـم طـريق عن السـابقة ،الضـوئي بـالمجهر وفحصـها الغـده من اجزاء علـى المجهر ، النســـيجيه ئيـهميـاالكـي المناعـه والطـرق والتحليـــل المورفولوجيـــة دراسة ،الإلكـــترونى مجهرية ،ملحوظ ضوء :النتـــائج :الإحصـــائي لوحظـت المورفولوجيـــة والتغـــيرات الإلكـــترون بنســـبة تحســـنت الـــتي الهرمونـات لنضـــوب نتيجـــة البـــديل الهـرموني.
فكيـــه التحـــت الغـدة بيـــن قويـــه علاقـة هناك :الخلاصـــة مثـل طرق بعـدة ذلـك رصـد تـم وقـد .المبيـــض ووظـائف الـرحم عنـق افـرازات باســـتخدام أو الجسـم حرارة درجة .دقيقـــه غـير الأســـاليب هذه تكـــون قـد ولكـن المخاطيـة