Original Article	**Effect of selective serotonin reuptake inhibitors (SSRIs) versus estradiol administration on anterior pituitary cell population and possible regenerative capacity in ovariectomized adult albino rats**

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ABSTRACT

Background: Reproductive ageing in women and the hormonal changes that occur during the onset of human menopause have been ascribed solely to ovarian failure and oocyte depletion. However, some studies have shown the central nervous system to be the major regulator of age-related reproductive dysfunction. Moreover, it has been postulated that the pituitary gland plays a key role in the menopausal process. Estradiol is a form of estrogen, a female sex hormone produced by the ovaries. Estradiol is used to treat symptoms of menopause such as hot flashes, and vaginal dryness, burning, and irritation. On the other hand, selective serotonin reuptake inhibitors (SSRIs) ease depression by increasing levels of serotonin in the brain.

Aim of work: Thus, it became the aim of the present study, to elucidate the effect of SSRIs versus estradiol in ovariectomized rats on the overall anterior pituitary cell population with special emphasis on the Folliculo-stellate (FS) cells.

Material and Methods: Thirty adult female Albino rats, ageing 3-4 months and 140-170 gm body weight were used in the present study. They were divided into 4 groups as follows:

Group A (control group): further subdivided into 2 subgroups each including 6 rats. A1: rats were left untreated throughout the experiment. A2: rats were left untreated then received 2ml subcutaneous (s.c.) sesame oil injection daily for one week prior to sacrifice. In the remaining 3 groups (6 rats each), the ovaries were ectomized and rats were left for five months to mimic estrogen-depletion models. Then; Group B: 6 rats were used as an ovariectomy model. Group C: 6 rats received 40µg/kg body weight estradiol by s.c. injection daily for one week prior to sacrifice. Group D: 6 rats received 10mg/kg fluoxetine at a single dose/day by feeding tube for one week prior to sacrifice.

Results: In the ovariectomized rats, there was loss of structural organization and an apparent decrease of cell population. Widespread vacuolations and cellular degeneration were also observed. An improved cellular architecture and cell population within pars distalis were seen with the administration of both estradiol and fluoxetine. Semithin sections confirmed these results. Immunostaining for the protein S-100 showed an apparent decrease in the distribution of the protein S-100 expression within the FS cells in ovariectomized rats that was significantly increased in response to estradiol and fluoxetine.

Conclusion: Ovariectomized rats revealed degeneration of the anterior pituitary cell population and decreased expression of protein S-100 in the pars distalis indicating decreased population of FS cells. Such effects were reversed by either estradiol or fluoxetine administration. This might suggest the added effect of both drugs if administered together on the improvement of anterior pituitary cell population as well as FC cells.

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Key Words: Ovariectomy, SSRIs, estradiol, menopause, pituitary, folliculo-stellate cells.

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INTRODUCTION:

Many women experience a variety of symptoms as a result of the hormonal changes associated with the transition to menopause. The hormonal changes associated with menopause actually begin prior to the last menstrual period, during a three to five year period sometimes referred to as the perimenopause. During this transition, women may begin to experience menopausal symptoms

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even though they are still menstruating. Follicle-stimulating hormone (FSH), released by the pituitary gland, is involved in controlling the menstrual cycle and the production of mature ovum by the ovaries. Researchers are wandering if this hormone, which peaks after menopause, may also play a role in an inflammatory process that leads to bone loss and blood vessel damage *(Radu et al. 2010)*. Despite some risks, estradiol replacement represents the classical therapeutic approach in the prevention of health problems due to ovarian hormone deficiency *(Mobasseri et al. 2004)*.

Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed to treat hot flashes affecting menopausal women. They may also help with irritability, depression, and moodiness. They can be used before and after menopause as a symptom treatment alternative to hormones as birth control pills or hormone therapy *(Freeman et al. 2011)*. SSRIs produce their therapeutic action and adverse effects by selectively binding to the serotonin (5-hydroxytryptamine, 5-HT) transporter, thereby blocking the reuptake of serotonin into pre-synaptic neurons, which results in enhanced serotonergic function in the brain *(Paulose-Ram et al. 2007)*.

The pituitary gland is often referred to as the "master gland" of the body, since it regulates many activities of other endocrine glands. All higher life forms critically depend on hormones being rhythmically released by the anterior pituitary. The proper functioning of this master gland is dynamically controlled by a complex set of regulatory mechanisms that ultimately determine the fine tuning of the excitable endocrine cells. Mechanisms of pituitary cellular plasticity are at present far from understanding *(Takuma et al. 1998; Solov'ev et al. 2008)*.

The anterior pituitary gland consists of various cell types, including five types of hormone-producing cells and folliculostellate (FS) cells, which do not produce classical anterior pituitary hormones *(Tsukada et al. 2014)*. Pituitary folliculostellate (FS) cells were originally described in 1953 and comprise up to 10% of the anterior pituitary cell population. Unlike their endocrine counterparts, they are devoid of secretory granules and are considered non-secretory until relatively recently. Experiments have demonstrated that FS cells play an important role in three broad areas of pituitary function: autocrine/paracrine regulation of anterior pituitary cell function via cytokines and growth factors, intrapituitary communication between various cell types, and modulation of inflammatory responses. (Vankelecom, 2007; Heinzlmann and Koves, 2008).

So, it become the aim of the present study, to elucidate the effect of SSRIs versus estradiol in ovariectomized rats on the overall anterior pituitary cell population with special emphasis on the Folliculo-stellate cells.

MATERIAL AND METHODS:

Animals:

Thirty adult female albino rats, ageing 3-4 months and weighing an average of 140-170 gm, were used in the current study. Animals were purchased from Research Unit and Bilharzial Research Center of Faculty of Medicine, Ain Shams University. Rats were maintained under routine conditions with free access to food and water, 12 hours light: 12 hours darkness. Rats were fed on standard rat diet and were allowed free water access. All experiments were carried out in accordance with the guide of the Committee of the Animal Research Ethics (CARE) - Faculty of Medicine- Ain Shams University.

Rats were divided into 4 groups (control group was further subdivided into 2 subgroups) as follows:

Group A: further subdivided into 2 subgroups each including 6 rats. A1: rats were left untreated throughout the experiment. A2: rats were left untreated then received 2ml subcutaneous (s.c.) sesame oil injection daily for one week prior to sacrifice.

In the remaining 3 groups, the ovaries were ectomized and rats were left for five months to mimic estrogen-depletion models *(Yang et al. 2009)*. Then;

Group B: 6 rats were used as an ovariectomy model.

Group C: 6 rats received 40µg/kg body weight estradiol by s.c. injection daily for one week prior to sacrifice (2ml of the diluted 1:1000 solution in sesame oil).

Group D: 6 rats received 10mg/kg fluoxetine at a single dose/day by feeding tube for one week prior to sacrifice.

Steps of ovariectomy *(Hamed et al. 2010)*:

A) Anaesthesia: The animals were

anaesthesized with intraperitoneal injection of thiopental sodium (40 mg/kg) then when surgical anaesthesia (judged by loss of withdrawal reflexes) had been reached, the animals were placed on the operating table. Surgical anaethesia was maintained throughout the operation by the use of a mask containing ether-soaked cotton wool.

B) Surgical procedure: A single longitudinal skin incision was made in the ventral midline of the abdomen above the symphysis pubis. The skin was then retracted laterally. The abdominal wall and the peritoneum were incised from the urinary bladder to the level of the lower poles of the kidneys. The ovary was exposed on each side. Haemostasis had been assured by ligation of the upper horns of the uterus with a cat gut thread. The ovary together with its surrounding fat and the oviduct was removed. The incision was closed with three to four interrupted stitches using silk threads and a curved needle.

Asepsis was maintained during the operation and antibiotic powder was sprinkled in the wound.

Drugs:

Folone (Estradiol Benzoate) in the form of 5mg/ml oily solution purchased from Misr Company for Pharmaceutical Industries was used in the present study. The drug was diluted in sesame oil 1:1000 and given in a dose of 40µg/kg body weight by s.c. injection for one week, this dose was calculated according to *Suzuki et al. (2006)*. Half of the control rats were left untreated, the other half received daily injections of the vehicle oil (sesame oil) for one week.

SSRI Prozac (Fluoxetine Hydrochloride; FLX), the product of Eli Lilly and Company, Indianapolis, USA, was used in the present study. Each commercially available capsule contains 20-mg FLX. 10mg fluoxetine /kg body weight were given to each fasted animal after being dissolved in distilled water at a single dose/day by intragastric feeding tube *(Erdemir et al. 2014)*.

Tissue extraction

At the end of the experiments, rats in the four groups were sacrificed by cervical dislocation which involves the separation of the cervical bone from the base of the skull or within the cervical spine area (the upper third of the neck).The rats were held at the cervical region by the left hand while the tail was drawn with the right hand dislocating the spinal column from the skull. The animals were placed in the anatomical position; the pituitary gland was then approached through the anterior cranial fossa, by breaking the bone of the skull around the orbit. The brain was accessed and the frontal lobe of the brain was raised. The pituitary gland was located below the brain between the optic nerves, lying on the sella turcica. The pituitary gland was then removed *(Ukoha et al. 2013)*.

Light microscopy

Half of the specimens were fixed in 10 % neutral formalin for one week then they were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin wax. Five µm thick sections were subsequently cut and stained with Hematoxylin & Eosin, then examined by the Olympus light microscope (Drury and Wallington, 1980).

The other half of specimens was immediately embedded in 2.5% phosphate-buffered glutaraldehyde (pH 7.3) at 4°C and specimens were cut into 1 mm cubes. Postfixation in 1% buffered osmium tetroxide for 1 to 2 hours was followed by dehydration in ascending grades of ethyl alcohol, then clearance in propylene oxide, and finally embedding in fresh epoxy resin. Semithin sections 1 µm in thickness were cut with a glass knife and stained with 1% Toluidine blue, and examined under light microscope (Bancroft and Gamble, 2002).

Immunohistochemistry for protein S-100:

Folliculo-stellate (FS) cells express and are immunostained with protein S-100. The streptavidin-biotin immunoperoxidase method was used. The tissue sections were first deparaffinized with xylene and hydrated by decreasing concentrations of ethanol. After incubation for 20 min in a solution of 3% H_2O_2 in water to inhibit endogenous peroxidase activity, the specimens were washed (3×10 min) (3 times, 10 min. each) in phosphate-buffered saline (PBS, 0.01 M, pH 7.4). Non-specific binding sites for immunoglobulins were blocked by 15 min-incubation with 0.25% casein in PBS, washed in PBS and incubated with the polyclonal primary antibody. The slides were subsequently washed (3×10 min) (3 times, 10 min. each) in PBS. The immunohistochemical visualization was carried out using the Ready-to-Use Immunostaining Kit (QD000-5L; BioGenex, San Ramon, Calif., USA) at 20°C. The sections were incubated for 30 min

with biotinylated anti-IgG and finally washed in PBS. The reaction site was revealed by 100 µL diaminobenzidine tetrahydrochloride (DAB) chromogen solution in 2.5 ml PBS and 50 µL H2O2 substrate solutions, resulting in a brown precipitate. The sections were counterstained with Hematoxylin *(Acosta et al. 2010)*.

Quantitative Image Analysis:

The measurements were done using the image analyzer (Image J program) in the Anatomy Department, Faculty of Medicine, Ain Shams University. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Each field was enclosed inside the standard measuring frame, and then the positively reacting protein S-100 immune-stained areas were masked by a blue binary color to be measured. The density (area percentage) of the immune-staining was measured in five fields from five serial sections from five animals from each group in accordance with *Gao et al. (2006)*.

Statistical Analysis:

Analysis of variance (ANOVA) and Bonferroni post hoc *t-test* were used to compare the positive protein S-100 immune-stained area percentage/ HPF in the four groups. The results were calculated as Mean + SD. *P-value* was calculated using the SPSS program. The significance of the data was determined by *P-value (P<0.05* or equal to 0.05 was considered significant and *P<0.001* or equal to 0.001 was considered highly significant) (Sawilowsky, 2005). *P-value* was corrected according to Bonferroni procedure by dividing the α value by the number of the compared groups (m) (α / m) so the significant *P-value* became *(P≤0.0125)* and highly significant *P-value* became *(P≤0.00025)* (Frane, 2015).

RESULTS:

A] Biochemical results:

Ovariectomy significantly decreased estrogen level [(11 ± 4) pg/ml, *P < 0.01*] as compared with control group [(21 ± 8) pg/ml], while estradiol administration increased the estrogen level [(63 ± 13) pg/ml, *P<0.01*] in blood. Fluoxetine didn't affect estrogen level compared to ovariectomy group [(10 ± 6) pg/ml, *P>0.01*.

On the other hand, ovariectomy caused significant increase in the level of FSH [(30.2 ± 2.7) miu/ml, *P<0.01*] as compared with control group [(5.6 ± 0.48) miu/ml], while estradiol administration decreased the FSH level [(7.1 ± 1.3) miu/ml, *P<0.01*] in blood compared to ovariectomy group (group B). Fluoxetine didn't significantly affect FSH level compared to ovariectomy group [(32.7 ± 1.9) miu/ml, *P>0.01*.

B] Histological results:

The anterior pituitary (also called the adeno-hypophysis or pars anterior or pars distalis), together with the posterior lobe (posterior pituitary, or the neurohypophysis) makes up the pituitary gland. The two lobes are separated by the hypophyseal cleft. Pars tuberalis and pars intermedia constitute two extensions from the anterior pituitary (Fig.1)

In the control group, examination of the pituitary gland of adult female control rat (A1 and A2) revealed similar findings. Hx and E stained sections showed that the cells of the pars distalis were organized in irregular clusters or cords separated by blood sinusoids. It contained two types of cells including chromophobe and chromophil cells. Chromophils appeared as large polygonal cells with round eccentric nuclei, while chromophobes were smaller cells arranged in groups with pale nuclei (Fig. 2). The cells were differentiated by their staining properties into acidophils, basophils or chromophobes. Both acidophils and basophils were large and polygonal in shape, with round eccentric vesicular nuclei. The acidophils stained red, while the basophils stained different shades of blue. Acidophils had a peripheral position compared to the more central position of the basophils. The chromophobes had rounded vesicular, relatively large nuclei and pale cytoplasm (Fig. 3).

Semithin sections of the pituitary gland of the control adult female albino rat showed the chromophils as large polygonal cells with round eccentric vesicular nuclei and a lot of granules in the cytoplasm. Many of the nuclei of chromphil cells had double nucleoli indicating high metabolic rate. Chromophobes were smaller with pale nuclei and absence of granules (Fig. 4).

Immunostaining for the S-100 protein was positive and revealed the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as dark brown spots or star-shaped cells. Most of the FS cells of pars distalis were immunostained with anti S-100 protein in both nucleus and cytoplasm. They were distributed throughout the parenchyma in relation to both endocrine cells and blood vessels (Fig. 5).

In the overectomized group B, loss of structural organization and an apparent decrease of cell population within pars distalis were common features. Widespread vacuolations and cellular degeneration with diffuse pyknosis, karyorrhexis and dissolved cytoplasm were observed. Many cells revealed a characteristic signet ring appearance (Figs. 6 & 7).

Semithin sections of the pituitary gland of an ovariectomized adult female albino rat confirmed previous findings and revealed widespread cellular degeneration of pars distalis with apoptotic pituicytes, pyknotic nuclei, karyorrhexis, scattered vacuoles and wide intercellular spaces. An apparent increased number of degranulated cells was observed (Fig. 8).

Immunostaining for the S-100 protein showed an apparent decrease in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as marked decrease in the dark brown spots (Fig. 9).

In the overectomized group C, examination of Hx and E stained sections of the pituitary gland of an ovariectomized adult female albino rat treated by estradiol showed an improved cellular architecture and cell population within pars distalis (Fig. 10). Chromophils appeared as large polygonal cells with round eccentric nuclei and stained cytoplasm, while chromophobes appeared smaller and arranged in groups with pale nuclei and unstained cytoplasm (Fig. 11).

Semithin sections also revealed an improved cellular architecture of pars distalis. Chromophils appeared with round eccentric vesicular nuclei and multiple nucleoli. Chromophobes were smaller cells with pale nuclei. The cells were separated by large congested blood sinusoids. Moreover, Mitotic figures and reappearance of granules were observed in many cells (Fig. 12).

Immunostaining for the S-100 protein showed an apparent increase in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis compared to group B (Fig. 13).

In the overectomized group D, examination of Hx and E stained sections of the pituitary glands of ovariectomized adult female albino rats treated by fluoxetine also revealed an improved cellular architecture and population of pars distalis. Chromophils and chromophobes were apparently increased (Fig. 14). Chromophils appeared as large polygonal cells with round eccentric nuclei and stained cytoplasm, while chromophobes appeared smaller cells in groups with pale nuclei and unstained cytoplasm. Mitotic figures were also observed within many cells. However, scattered vacuolations and some cells with karyorrhexis were still present (Fig. 15).

Semithin sections also revealed an improved cellular architecture of pars distalis. Mitotic figures and reappearance of granules were observed in many cells. But some cells with pyknotic nuclei and scattered vacuoles were still present (Fig. 16).

Immunostaining for the S-100 protein showed an apparent increase in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis (as in group C) as compared to group B, seen as dark brown spots or stars (Fig. 17).

C] Quantitative image analysis and statistical results:

Quantitative image analysis and ANOVA statistical analysis revealed that there was a highly significant difference in positive protein S-100 immunostaining area percentage/HPF ($P < 0.001$) among the four groups (Table 1; Bar chart 1).

Bonferroni corrected post hoc test (t-test) for ANOVA revealed statistically highly significant decrease in the mean of area fraction % (positive protein S-100 immunostaining area percentage / HPF) among group B (Rats subjected to ovariectomy) *($P<0.00025$)* compared to the control group (Table II).

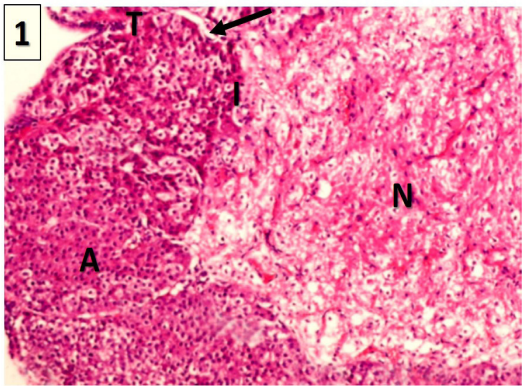


Fig. 1: Photomicrograph of the pituitary gland of a control adult female albino rat showing its two lobes namely, anterior lobe or adenohypophysis (A) and posterior lobe or neurohypophysis (N) separated by hypophyseal cleft (arrow). Note pars tuberalis (T) & pars intermedia (I). Hx & E x 40

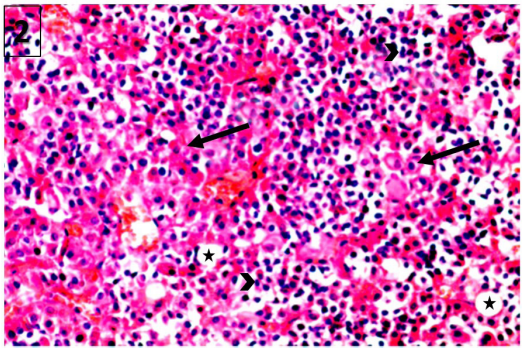


Fig. 2: Photomicrograph of the pituitary gland of a control adult female albino rat showing irregular cords of epithelial cells forming pars distalis. Chromophils (arrows) are large polygonal cells with round eccentric nuclei and stained cytoplasm while chromophobes (arrow heads) are smaller cells arranged in groups with pale nuclei and unstained cytoplasm. Cells are separated by large blood sinusoids (*).Hx & E x 400

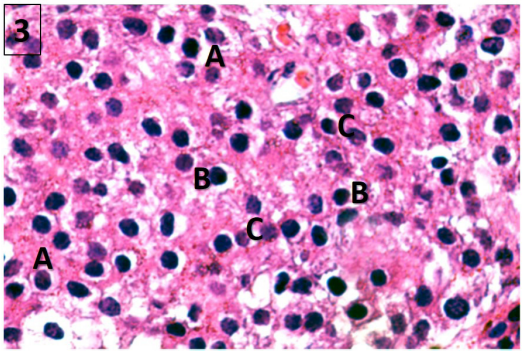


Fig. 3: Photomicrograph of the pituitary gland of a control adult female albino rat showing different types of cells forming the pars distalis. Acidophils (A) appear as large polygonal cells with round eccentric vesicular nuclei and acidophilic cytoplasm while basophils (B) appear as large polygonal cells with round eccentric vesicular nuclei and basophilic cytoplasm. Chromophobes (C) are smaller cells arranged in groups with pale nuclei and unstained cytoplasm. Note the more peripheral position of acidophils & central position of the basophils. Hx & E x 1000

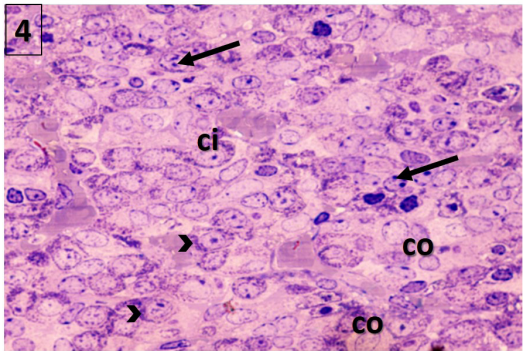


Fig. 4: Photomicrograph of a semithin section of the pituitary gland of a control adult female albino rat showing the chromophils (ci) as large polygonal cells with round eccentric vesicular nuclei and a lot of granules (arrow heads). Chromophobes (co) are smaller cells with pale nuclei and absence of granules. Note the nuclei of chromphil cells with double nucleoli (arrows). Toluidine blue x 1000

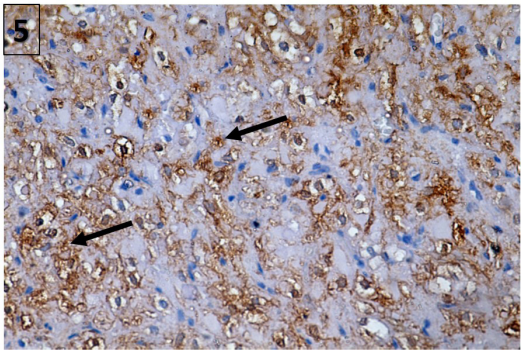


Fig. 5: Photomicrograph of the pituitary gland of a control adult female albino rat stained with protein s 100 immunohistochemistry showing the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as dark brown spots or star-shaped cells (arrows). Protein S-100immunohistochemistry x 400

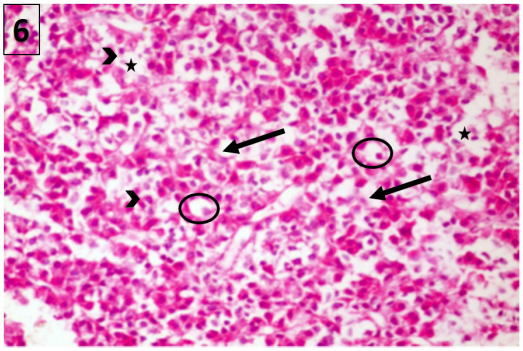


Fig. 6: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat showing loss of structural organization and an apparent decrease of cell population within pars distalis. Note the widespread vacuolations (arrows), cellular degeneration with diffuse pyknosis (arrow heads), dissolved cytoplasm (*) and the characteristic signet ring appearance in some cells (circles). Hx & E x 400

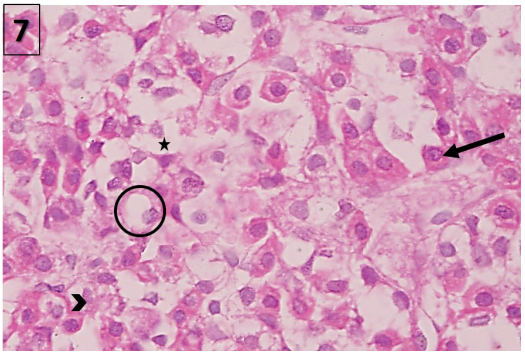


Fig. 7: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat showing widespread vacuolar degeneration of pars distalis and cellular degeneration with pyknotic nuclei (arrows), karyorrhexis (arrow heads), dissolved cytoplasm (*). Many cells revealed a characteristic signet ring appearance (circles). Hx & E x 1000

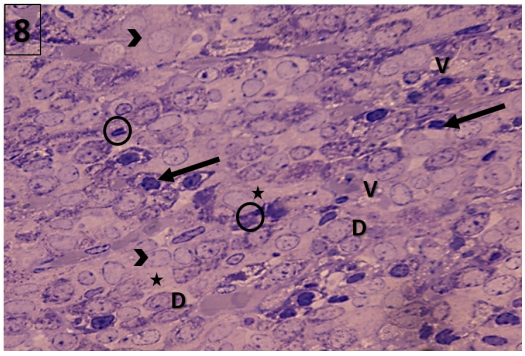


Fig. 8: Photomicrograph of a semithin section of the pituitary gland of an ovariectomized adult female albino rat showing widespread cellular degeneration of pars distalis with apoptotic pituicytes (arrow heads), pyknotic nuclei (arrows), karyorrhexis (circles) vacuoles (v) and wide intercellular space (*). An apparent increased number of degranulated cells (D) is observed. Toluidine blue x 1000

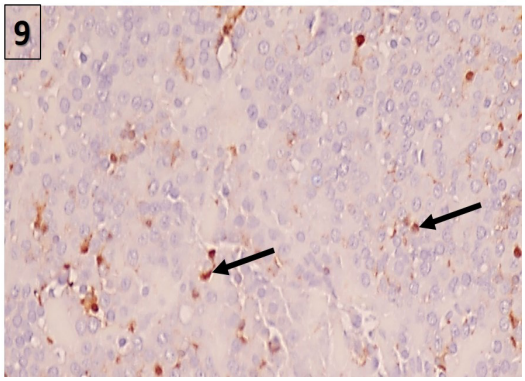


Fig. 9: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat stained with protein S-100 immunohistochemistry showing an apparent decrease in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as dark brown spots (arrows). Protein S-100 immunohistochemistry x 400

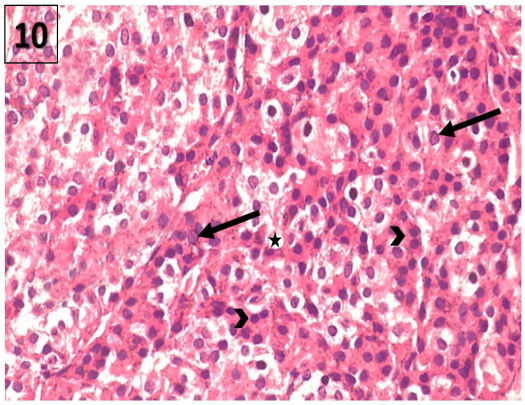


Fig. 10: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by estradiol showing improved cellular architecture and cell population within pars distalis. Chromophils (arrows) and chromophobes (arrow heads) are observed. Hx & E x 400

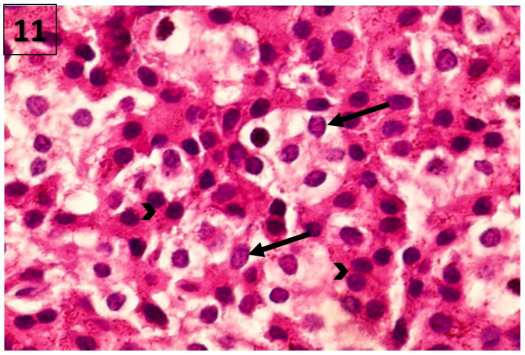


Fig. 11: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by estradiol showing improved cellular architecture of pars distalis. Chromophils (arrows) appeared as large polygonal cells with round eccentric nuclei and stained cytoplasm while chromophobes (arrow heads) are smaller cells arranged in groups with pale nuclei and unstained cytoplasm.

Hx & E x 1000

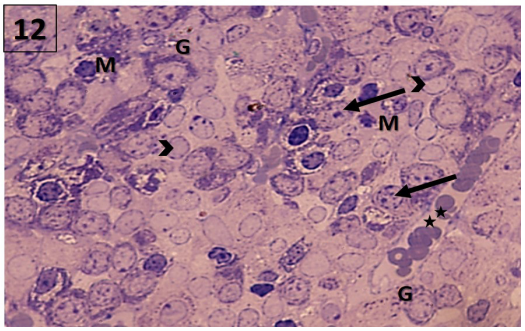


Fig. 12: Photomicrograph of a semithin section of the pituitary gland of an ovariectomized adult female albino rat treated by estradiol showing improved cellular architecture of pars distalis. Chromophils appeared with round eccentric vesicular nuclei and multiple nucleoli (arrows). Chromophobes are smaller cells with pale nuclei (arrow heads). The cells are separated by large congested blood sinusoids (**). Mitotic figures (M) and reappearance of granules (G) are observed in many cells. Toluidine blue x 1000

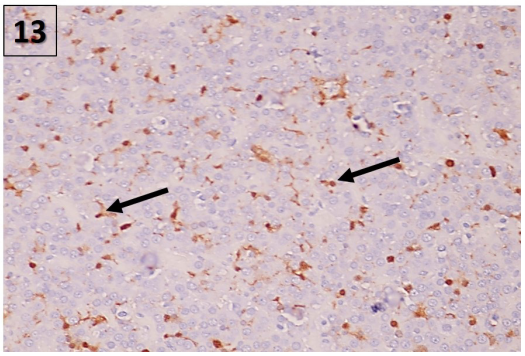


Fig. 13: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by estradiol, stained with protein S-100 immunohistochemistry showing an apparent increase (compared to group B) in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as dark brown spots (arrows).

Protein S-100 immunohistochemistry x 400

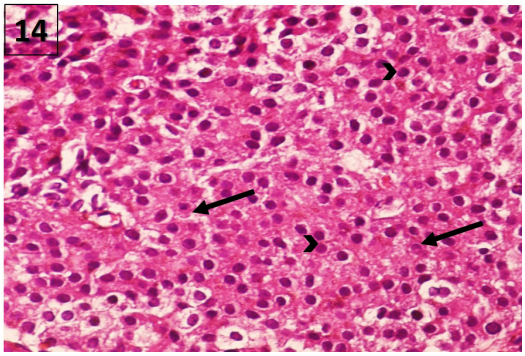


Fig. 14: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by fluoxetine showing an improved cellular architecture and population of pars distalis. Chromophils (arrows) and chromophobes (arrow heads) are apparently increased. Hx & E x 400

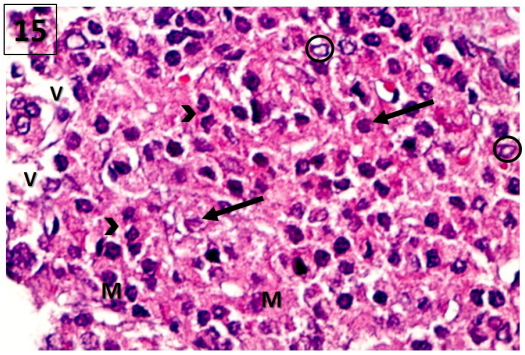


Fig. 15: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by fluoxetine showing an improved cellular architecture of pars distalis. Chromophils (arrows) appeared as large polygonal cells with round eccentric nuclei and stained cytoplasm while chromophobes (arrow heads) are smaller cells arranged in groups with pale nuclei and unstained cytoplasm. Mitotic figures (M) are observed in many cells. However scattered vacuolations (V) and some cells with karyorrhexis (circles) are stillpresent. Hx & E x 1000

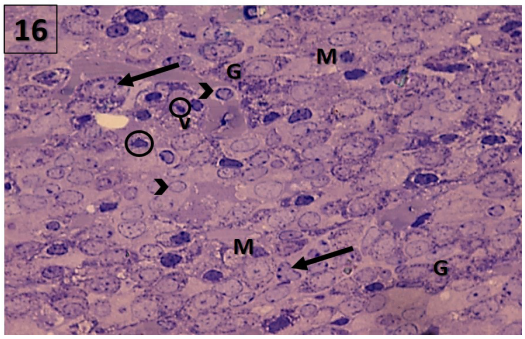


Fig. 16: Photomicrograph of a semithin section of the pituitary gland of an ovariectomized adult female albino rat treated by fluoxetine showing an improved cellular architecture of pars distalis. Chromophils appeared with round eccentric vesicular nuclei and multiple nucleoli (arrows), while chromophobes are smaller cells with pale nuclei (arrow heads). Mitotic figures (M) and reappearance of granules (G) are observed in many cells. Some cells with pyknotic nuclei (circles) and scattered vacuoles (V) are present. Toluidine blue x 1000

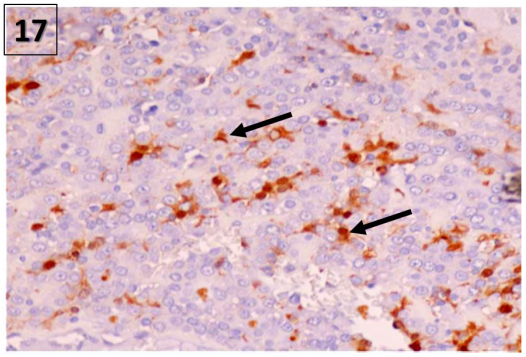
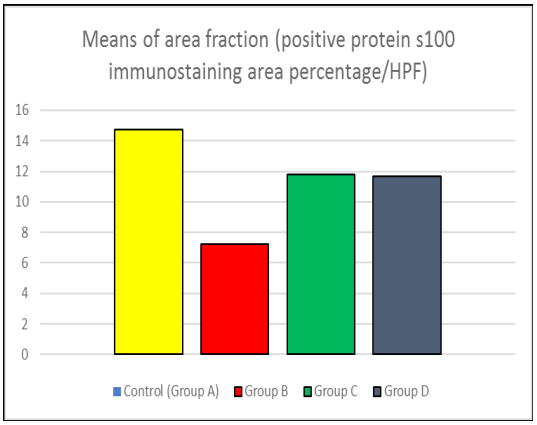


Fig. 17: Photomicrograph of the pituitary gland of an ovariectomized adult female albino rat treated by fluoxetine, stained with protein S-100 immunohistochemistry showing an apparent increase (compared to group B) in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis seen as dark brown spots or stars (arrows).

Protein S-100 immunohistochemistry x 400



Bar chart 1: Means of area fraction (%) (Positive protein S-100 immunostaining area percentage / HPF)

Table II: Means of area fraction (positive protein s100 immunostaining area percentage / HPF) in the control & group B (Bonferroni post hoc test)

	Control (Group A)	Group B
Mean	14.732	7.2536
Variance	0.078422	0.1725468
Observations	5	5
Pooled Variance	0.1254844	
Hypothesized Mean Difference	0	
df	8	
t Stat	33.37980735	
P(T<=t) one-tail	3.54104E-10	
t Critical one-tail	1.859548038	
P(T<=t) two-tail*	7.08209E-10	
t Critical two-tail	2.306004135	

**P-value* < *0.00025* (i.e. Highly significant)

Table I: Means of area fraction (positive protein S-100 immunostaining area percentage / HPF) in the four groups (ANOVA single factor)

Groups	Count	Sum	Average	Standard deviation ±	Variance		
Control (Group A)	5	73.66	14.732	0.280039283	0.078422		
Group B	5	36.268	7.2536	0.41538753	0.172547		
Group C	5	59	11.8	0.391136805	0.152988		
Group D	5	58.422	11.6844	0.711377748	0.506058		
Source of Variation	SS	df	MS		F**	*P-value**	F crit**
Between Groups	142.6575766	3	47.55252553		209.0186	4.85E-13	3.238872
Within Groups	3.6400604	16	0.227503775				
Total	146.297637	19					

*P-value among all groups **F value was greatly higher than F critical value

Table III: Means of area fraction (positive protein S-100 immunostaining area percentage / HPF) in the group C compared to group B (Bonferroni post hoc test)

	Group B	Group C
Mean	7.2536	11.8
Variance	0.1725468	0.152988
Observations	5	5
Pooled Variance	0.1627674	
Hypothesized Mean Difference	0	
df	8	
t Stat	-17.81779403	
P(T<=t) one-tail	5.0401E-08	
t Critical one-tail	1.859548038	
P(T<=t) two-tail*	1.00802E-07	
t Critical two-tail	2.306004135	

**P-value* < *0.00025* (i.e. Highly significant)

Table IV: Means of area fraction (positive protein S-100 immunostaining area percentage / HPF) in the group D compared to group B (Bonferroni post hoc test)

	Group B	Group D
Mean	0.1725468	0.5060583
Variance	5	5
Observations	0.33930255	
Pooled Variance	0	
Hypothesized Mean Difference	8	
df	-12.02703541	
t Stat	1.0537E-06	
P(T<=t) one-tail	1.859548038	
t Critical one-tail	2.10741E-06	
P(T<=t) two-tail*	2.306004135	
t Critical two-tail		

**P-value* < *0.00025* (i.e. Highly significant)

DISCUSSION:

Female ageing represents the biological process of structural and functional changes in endocrine cells and tissues, as well as in pituitary hormone-producing cells (Milosevic and Ajdzanovicthe, 2014). The anterior pituitary pars distalis is made up of many cell types that are essential for various physiological processes as growth, development, homeostasis, metabolism, and reproduction. Each hormone of the pars distalis is generally secreted by a separate cell type, but some cells can secrete two hormones (Knobil and Neill, 1994; *Yeung et al. 2006)*. Understanding of postmenopausal condition is hindered by the difficulty of studying a disease that is restricted to humans. Therefore, the use of an animal model provides more experimental material and allows assessment of possible therapies (Horndike and Turner, 1998). In the present study, ovariectomized rat model was chosen to clarify the menopausal effect on pars distalis. The ovariectomized (OVX) rat model is the approved preclinical model by the Food and Drug Administration (FDA) for studying the decline in endogenous estrogen production by the ovaries (Johnston and Ward, 2015).

The present work revealed that, ovariectomy significantly decreased estrogen level in blood, and caused significant increase in level of FSH as compared with control group, while estradiol administration increased the estrogen level in blood. Fluoxetine didn't affect estrogen level compared to ovariectomy group.

In the rat, ovarian estrogen regulates synthesis and release of gonadotrophins acting on intracellular estrogen receptor (ER) in the gonadotrope. Removal of estrogen by ovariectomy (OVX) in the rat induces profound effects on the synthesis and release of gonadotrophins *(Sanchez-Criado et al. 2006).*

In the control group of the present study, Hx and E stained sections showed that the cells of the pars distalis were organized in irregular clusters or cords separated by blood sinusoids. It contained two types of cells including chromophobe and chromophil cells. Chromophils appeared as large polygonal cells with round eccentric nuclei, while chromophobes were smaller cells arranged in groups with pale nuclei. Chromophils were large and polygonal in shape, with round eccentric vesicular nuclei. *Wheater et al. (1987)* described the cells of pituitary gland to be divided into chromophils (both acidophils, basophils) and chromophobes. The authors demonstrated that acidophils stain with Eosin and secrete Growth Hormone (GH) and are known as somatotrophs; or secrete ProLactin (PrL) and are known as lactotrophs. Some cells, however, can secrete both hormones and are called somatomammotrophs. On the other hand, the authors showed that basophils stain relatively poor, but have a slightly basophilic appearance in Hx&E stained sections. They generally secrete TSH (thyrotrophs) or FSH and LH (gonadotrophs) and to a lesser extent ACTH (adrenocorticotrophs). Chromophobes stain poorly with Hx&E and are characteristic of ACTH (corticotroph) secreting cells.

In addition, semithin sections of the present study showed the chromophils as large polygonal cells with round eccentric vesicular nuclei and a lot of granules. Many of the nuclei of chromphil cells had double nucleoli indicating high metabolic rate. Chromophobes were smaller with pale nuclei and absence of granules. Greaves (2007) explained high metabolic activity of chromophils and added that basophils and eosinophils that have recently degranulated or are in the process of active synthesis of hormone might appear even chromophobic.

Moreover, in current work, acidophils were seen located predominantly in the lateral aspects of the lobes while basophils appeared more concentrated in the central portion of the pars distalis. These findings are in accordance with Greaves (2007).

Group B, in the present work, that were left without treatment for five months following the ovariectomy, showed loss of structural organization with an apparent decrease of cell population within pars distalis. *Ronchetti et al. (2016)* explained that changes in the estrogenic status produce deep changes in pituitary physiology, mainly because estrogens (E2) are one of the main regulators of pituitary cell population. The authors also added that cell activity gradually decreased after ovariectomy (OVX) and lowered E2 levels because of cell death.

Also, the present work revealed widespread vacuolations and cellular degeneration with diffuse pyknosis, karyorrhexis and dissolved cytoplasm of pituicytes in both Hx and E and semithin sections. Apoptosis normally occurs during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (Norbury and Hickson, 2001). Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis (Elmore, 2007).

Furthermore, an apparent increased number of degranulated cells and cells with characteristic signet ring appearance were seen in the present study. This was in accordance with Abd El-Maksoud and Moustafa (2003) who observed that cells of pars distalis of aged female rats had little secretory granules and many vacuoles in the semithin sections after ovariectomy.

Capen (1983) described that the effects seen in the hormone secreting cells of the pars distalis, following gonadectomy were time dependant. At first, the author noticed rapid release of the storage granules from the affected trophic hormone secreting cells. This was followed by increased size of the degranulated cells due to expansion of their cytoplasm. Those affected cells become vacuolated due to cystic dilation of the rough endoplasmic reticulae. Finally, the small vacuoles will coalesce into a single large clear vacuole that often displaces the nucleus peripherally and forms the characteristic “signet ring” cell.

The clear observation of an apparent decrease in anterior pituitary cell population detected in the ovariectomized (group B) rats and its apparent regain following estradiol administration in group C of the present work, was also recorded by *Brown et al, (2004)*. The authors explained that steroid hormones are known to increase the secretory ability of cells. They also added that adult ovariectomized female rats receiving hormone replacement therapy for 10–14 days showed an increase in the number of lactotrophs and increased amounts of PRL that were secreted by individual lactotrophs.

Moreover, in the present work, both Hx and E and semithin sections of group C that was treated by estradiol, showed an improved cellular architecture and cell population within pars distalis, with the appearance of mitotic figures and secretory granules in many cells. Seilicovich (2010) demonstrated that regulation of tissue remodeling in the anterior pituitary is closely related to changes in hormonal status. The author explained that the number of anterior pituitary cells, in the rat, fluctuates depending on different physiological situations, especially in response to alterations in estrogen levels after ovariectomy, given that these hormones are significant in the regulation of pituitary cell populations and that estrogens exert proliferative actions on this gland.

In addition, in current work, the histological stained sections of the pituitary gland of an ovariectomized adult female rat treated by fluoxetine also revealed an improved cellular architecture and cell population of pars distalis. Number of cells apparently increased and mitotic figures were observed in many cells. However scattered vacuolations and some cells with karyorrhexis were still present. SSRIs including fluoxetine, selectively inhibit membrane associated serotonin transporter thus inhibits serotonin re-uptake. Increased synaptic availability of serotonin stimulates large number of receptor subtypes which lead to complex secondary responses *(Aggarwal et al. 2016)*.

Benmansour et al. (2016) clarified a comparable antidepressant like effects of estradiol versus SSRIs on middle aged ovariectomized rats and concluded that SSRIs have identical effects on the serotonin transporter in females regardless of age and the time of treatment initiation after menopause. However, if estradiol is to be tried in postmenopausal women, it might only be helpful to those in perimenopause or shortly after menopause begins.

On the other hand, in the present work, immunostaining for the protein S-100 in the control group was positive and revealed the normal distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis. Folliculo-stellate cells appeared as dark brown spots or star-shaped cells. Most of the FS cells of pars distalis were immunostained with anti-protein S-100 in both nucleus and cytoplasm. They were distributed throughout the parenchyma in relation to both endocrine cells and blood vessels.

Tissue maintenance in adults requires a constant supply of new cells to replace differentiated cells lost to stress and damage or destroyed as a part of normal cell death program. Several different cell types, including folliculostellate cells of the anterior pituitary have been recently proposed as stem cells *(Gleiberman et al. 2008)*. *Yoshida et al. (2011)* suggested that FS cells have a wide differentiation capacity to other anterior pituitary cell population like organ specific stem cells. This might contribute to a self renewal system in the pars distalis of the pituitary gland *(Osuna et al. 2012)*.

Moreover, functions ascribed to FS-cells include the formation of an extensive and complex tri-dimensional network; scavenger activity by engulfing degenerated cells; regulation of endocrine cells by producing various growth factors, cytokines; and large-scale inter-cellular communication by means of their long cytoplasmic processes and gap junctions. The FS-cell is known to be positive for protein S-100 (Devnath and Inoue, 2008). The distribution of the protein S-100 cells (folliculo-stellate cells) is very important for understanding of the regulation of the anterior pituitary *(Sato et al. 2005)*.

There is an increasing evidence for an intra-pituitary communication system by which information is transferred via the network of non-endocrine folliculo-stellate cells. Local electrical stimulation of FS cells triggers cytosolic calcium waves, which propagate to other FS cells by signaling through gap junctions. FS cell coupling could relay information between opposite regions of the gland and provide an efficient mechanism that masters anterior pituitary functioning in response to physiological needs *(Fauquier et al. 2001; Mehet et al. 2012)*.

Immunostaining for protein S-100 in current study, revealed an apparent decrease in the distribution of protein S-100 expression within the folliculo-stellate cells of pars distalis of ovariectomized rats. On the contrary, this distribution was apparently increased in response to estradiol as well as fluoxetine. Moreover, quantitative image analysis and statistical studies in the present work approved that there was a highly significant decrease in the mean of area fraction percentage among group B compared to the control group. Quite the reverse, there was a statistically highly significant increase in the mean of area fraction percentage among both group C *($P<0.00025$)* and group D *($P<0.00025$)* compared to group B.

The findings of the present work were in accordance with *Cónsole et al. (2000); Yamashita et al. (2005)* who measured the cell density of FS cells in S100-reacting elements by means of an image analysis system (Imaging Technology, Optimas) and suggested a significant decrease *($p<0.05$)* in FS cells in both old and senescent rats as compared to young animals.

FS cells seem to be targeted by estrogen because they have both estrogen receptor α and estrogen receptor ß (Horvath, and Kovacs, 2002). Estrogen receptors, both α and ß, are present in subpopulations of rat pituitary cells, particularly lactotrophs and a proportion of folliculo-stellate cells (Nolan and Levy, 2009). In addition, glucocorticoid receptor (GR) immunoreactivity has been reported in folliculo-stellate (FS) cells in the rat anterior pituitary gland. These cells might be directly or indirectly influenced by glucocorticoids via GR in order to regulate cell functions, including the synthesis and/or secretion of hormones *(Ozawa et al. 2002)*. This might explain that ovariectomy affected the number of FS cells due to a significant decrease in blood corticosterone that was reversed by the administration of estradiol *(Davies et al. 2007)*.

Also, the selective serotonin reuptake inhibitor, fluoxetine, increases protein S-100 content and stimulates protein S-100 secretion in the serotonergic neurons *(Baudry et al. 2010)*. Serotonergic neurons play an important role in the regulation of neuroendocrine function carried out via both sympatho-adrenal and hypothalamic-pituitary adrenal (HPA) pathways *(Briscoe et al. 2008)*.

Moreover, the universal measures for the treatment of postmenopausal symptoms are antidepressant therapy and hormone replacement therapy. Many contraindications to this hormone replacement therapy and the potential risk of cancer as well as many side effects limit their use in clinical application *(Huang et al. 2015)*. On the other hand, the antidepressant-like effect of estradiol and fluoxetine in young adult and middle-aged female ovariectomized rats is well established with menopausal depression. These effects might lead to several studies on the use of their synergistic effect *(Récamier-Carballo et al. 2012)*.

In conclusion, the present study on menopausal model of ovariectomized rats revealed degeneration of the anterior pituitary cell population and decreased expression of protein S-100 in the pars distalis indicating decreased population of FS cells. Such effects were reversed by either estradiol or fluoxetine administration. This might suggest the added effect of both drugs if administered together on the improvement of anterior pituitary cell population as well as FS cells. Further research will be conducted on the ultrastructural changes in the FS cells in response to ageing or menopause and the impact of these changes on the cell functions.

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تأثير مثبطات امتصاص السيروتونين الانتقائية مقابل الاستراديول على خلايا الغده النخامية الأمامية واحتماليه القدرة على التجدد في الجرذان البيضاء البالغة مستأصلي المبيض

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ملخص البحث

المقدمة: أرجعت اسباب الشيخوخة الإنجابية لدى النساء والتغيرات الهرمونية التي تحدث أثناء بداية سن اليأس البشري فقط لفشل المبيض واستنزاف البويضات. وبالرغم من ذلك، فقد أظهرت بعض الدراسات ان الجهاز العصبي المركزي هو المنظم الرئيسي لضعف الإنجاب المرتبط بالعمر. وعلاوة على ذلك، فقد افترض أن الغدة النخامية تلعب دورا رئيسيا في عملية انقطاع الطمث. يعتبر الاستراديول شكل من أشكال هرمون الاستروجين، وهو هرمون الإناث الذي ينتجه المبيض. يستخدم الاستراديول لعلاج أعراض انقطاع الطمث مثل الهبات الساخنة، وجفاف المهبل، والحرقان، والتهيج. من ناحية أخرى، تعمل مثبطات امتصاص السيروتونين الانتقائية SSRIs على تخفيف الاكتئاب عن طريق زيادة مستويات السيروتونين في المخ.

الهدف من البحث: وهكذا، فإنه اصبح الهدف من هذه الدراسة هو توضيح تأثير ال SSRIs مقابل الاستراديول على خلايا الجزء الأمامي للغدة النخامية، مع التركيز بشكل خاص على خلايا الجريبات النجمية .(FS)

المواد والطرق المستخدمة: استخدم فى هذه الدراسه ثلاثون من اناث الجرذان البالغين، تتراوح اعمارهم من 3-4 شهور واوزانهم ما بين 140-170 جرام من وزن الجسم. تم تقسيمهم إلى 4 مجموعات على النحو التالي:

المجموعة A: المجموعة الضابطة: قسمت الى مجموعتين 6) A1 جرذان) تركت دون علاج و6) A2 جرذان) تركت دون علاج ثم اعطيت 2 مل من زيت السمسم تحت الجلد يوميا لمدة أسبوع واحد قبل التضحية بهم. بقية المجموعات (6 فئران في كل مجموعة) تم استئصال المبايض ومن ثم تركوا لمدة خمسة أشهر لتقليد نماذج نضوب الاستروجين. المجموعة B: 6 جرذان استخدمت كموديل لاستئصال المبايض. المجموعة C: 6 جرذان تلقت 40 ميكروغرام / كجم استراديول حقن تحت الجلد يوميا لمدة أسبوع واحد قبل التضحية بهم. المجموعة D: 6 جرذان تلقت 10مجم / كغ فلوكستين بجرعة واحدة يوميا عن طريق أنبوبه معديه لمدة أسبوع واحد قبل التضحية بهم.

النتائج: في الجرذان التى تم فيها استئصال المبايض كان هناك فقدان التنظيم الهيكلي وانخفاض واضح في اعداد الخلايا. وقد لوحظت انتشارات واسعة النطاق من الانحطاط الخلوي. وقد شوهدت بنية خلوية محسنة وزيادة في اعداد الخلايا داخل pars distalis مع استخدام كل من الاستراديول والفلو كستين. وأكدت المقاطع نصف الرقيقة هذه النتائج. كما أظهرت الدراسة المناعية للبروتين S-100 انخفاضا واضحا في توزيعه داخل خلايا الجريبات النجمية في الفئران المستأصلة المبايض وزادت بشكل كبير استجابة للاستراديول والفلو كستين.

الخاتمه: كشفت هذه الدراسة انحطاط اعداد الخلايا النخامية الأمامية وانخفاض التعبير عن بروتين S-100 مما يشير إلى انخفاض عدد خلايا. الجريبات النجمية. تم عكس هذه الآثار عن طريق الاستراديول أو الفلو كستين. وهذا قد يشير إلى تأثير إضافي على حد سواء إذا تم اعطاؤهم معا على تحسين الخلايا النخامية الأمامية وكذلك خلايا الجريبات النجمية.