Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Morphological and Hormonal Changes of Pituitary-Gonadal Axis in Domestic Queens at Different Estrous Phases

Mohammad S. Jawad[1]; Jabbar A. A. Al-Saaidi[2]
[1]Dept. Physiology, College of Veterinary Medicine, University of Al- Qadisiyah, Al-Qadisiyah, Iraq.
[2]Dept. Physiology, College of Veterinary Medicine, University of Al- Qadisiyah, Al-Qadisiyah, Iraq.
Corresponding Author Email: jabbar.alsaadi@qu.edu.iq.
ORCID ID: 0000-0003-2022-2287.
Received:12/2/2023. Accepted:9/3/2023. Published:1/6/2023

Abstract

This study aimed to evaluate the morphological changes of pituitary, ovarian, and uteri and the expression levels of ovarian *fshr*, *lhr*, *inhα*, and *cyp19a* genes at different estrous phases. Ten adult queens, at each estrous phase, were included. The pituitary glands, ovaries, and uteri were dissected for histological examination and molecular analysis. Higher ovarian weight at the estrus phase and higher uterin weight at the diestrus phase were shown compared with other estrous phases. Ovarian growing follicle diameter, myometrium thickness, pituitary gland dimeter, and the expression levels of ovarian *fshr*, *lhr*, *inhα*, *cyp19a* genes significantly elevated at the estrus and proestrus phases among other phases. At the proestrus phase, the ovarian sections showed different stages of follicular growth, whereas the estrus phase showed disintegrated tertiary follicles and hypertrophied granulosa and theca cells. The diestrus phase revealed the formation of corpus luteum and atresia follicles, while the anestrus phase showed the different types of follicles. The uteri sections at the proestrus and estrus phases revealed striated and lengthening uterine glands and increased vascularization. At the diestrus phase, the uteri showed reduced vascularization, whereas those at the anestrus phase showed reduced uterine glands development. The pituitary sections at the proestrus and estrus phases revealed differential staining characteristics and sizes of secretory granules compared with diestrus and anestrus phases. It could be concluded that significant histological and functional changes occure in the pituitary-gonadal axis at different estrous phases of adult queens.

Keywords: Queen, Reproduction, Pituitary gland, Ovary, Uterus

Introduction

The cat is a small carnivorous mammal that is valued by human for its companionship and its ability to hunt household pests, and is currently the most popular pet in the world [1]. The queen's reproductive system is quite different from the reproductive systems of many animals, as the queen normally enters puberty between the ages of 5 and 12 months, with the precise timing dependent on the length of the present photoperiod and its weight [2]. The queen is periodically polyestrous, which means that she has multiple ovulatory cycles at the time of year when she is supposed to be reproducing, but she goes through a protracted period of anestrus during the darker and colder months of the year [3]. In most cases, the breeding season starts in January or February and lasts until the end of

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

summer or the beginning of autumn [4]. As a result of copulation generating the production of luteinizing hormone (LH), which ultimately results in ovulation, the female cat is believed to be an induced ovulatory [5]. This is the case for the vast majority of queens. However, it has been shown that spontaneous ovulation may happen randomly in as much as sixty percent of all queens [6].There are four stages that make up the ovarian cycle of a queen: proestrus, estrus, diestrus, and anestrus. The estrous cycle in the unbred cats during two weeks and estrus may last anywhere from three to seven days. This length is determined by the regular changes in estrogens [7]. The proestrus phase may extend for one to two days, which is characterized by the existence of ovarian follicles that are between one and two millimeters in diameter and the production of estradiol-17ß in high quantities throughout the body which is associated with a high speed of the follicular growth. It is the stage in which attracted male to the female's non-receptive [8].The queen's estrus, also termed as a behavioral stage of receptivity to mating or breeding period, so this stage is detected solely by queen's behavioral response to the male, its begins when she allows the male to mount and finished when this behavior cease [2]. Estrus may last anywhere from three to sixteen days for a female, and during this period, she will exhibit evident mating activities such as calling, rolling, and peeing. The estradiol-17ß increased in the serum, which depended on the follicular growth. This leads to a quicker onset of estrous behaviour. Around day 3, the behavioural indicators are at their most pronounced in association with the peak estrogen secretion. Preovulatory follicles that are distinct (with diameters more than 2 millimeters; typically (1-4) follicles for one ovary protrude on the ovary surface. Estrus lasts for the same amount of time regardless of female whether or not has engaged in sexual activity or ovulated [7]. Simpson *et al*. [8] suggested that after estradiol-17ß rise if no ovulation occurs, it will decrease to basal concentration within 5-10 days. If ovulation occurs, the concentration will decline within 2-3 days.The diestrus phase is the time in which progesterone is the dominant hormone in the queen [9]. After ovulation without pregnancy, the diestrus will follow [10]. Corpora lutea will form in this phase within 24 -48 hours of ovulation and begin secreting progesterone [11]. They remain functional for 30-50 days (average 35 days) in nonpregnant queens, at which time regression occurs, and an interestrus interval follows [10]. When ovulating queens reach diestrus, they stop displaying estrual postures and become inattentive to the male. As soon as 64 hours have passed from the initial mating, the cytoplasm of the granulosa and theca interna cells of the corpora hemorrhagica begins to vacuolate, also known as luteinizing. Within the first 48–72 hours following ovulation, the CL will grow and there is a rise in circulating progesterone that is greater than the baseline (40 h after ovulation), after which they begin to rise in tandem with peripheral progesterone. After increased progesterone levels, in queens that are not pregnant, CL begins to progressively regress, resulting in the formation of chronic luteal scars. After this, the majority of females will recommence their estrous cycle within one week to two weeks [7].The anoestrus is defined as the period of clinical reproductive quiescence and a seasonal absence of cycling activity [2]. Long [12] detailed that the anoestrus is the period during the winter when there is no ovarian activity. Atresia occurs in the ovary, and the estradiol-17ß levels are at their lowest point. In cycling females who do not ovulate, the average length of the interestrous phase is anything from 7 to 21 days. Despite this, many cats seem to cycle continues throughout the year [7]. Hormonally, the anoestrus period is due to the production of melatonin and the suppression of GnRH

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

production from the hypothalamus. Thus the anterior pituitary gland does not release the gonadotropins (FSH and LH) [13]. This will remain the concentration of the estrogen and progesterone basal levels [8,9].The current study was performed to evaluate the morphological and endocrinological changes of pituitary-gonadal axis in adult queens that associated with different estrous phases**.**

Materials and Methods

Experimental animals: Domestic adult queens (aged more than one year) were were captured by feral cat traps at different phases of the estrous cycle to be included in the current study.

Experimental protocol: Ten adult queens at each of the proestrus, estrus, diestrus, and anestrus phases were captured, anaesthetized with ketamine (90 mg/kg bw) and xylazine (40 mg/kg bw), and euthanized (the animal handling was according to the international and national animal welfare standards). The pituitary glands, ovaries and uteri were dissected and fixed in 10% formalin for the histological examination. Ovarian tissue samples were taken and immediately stored in DEPC for molecular analysis.

Preparation, staining, and examination of the histological sections: The formalin fixed specimens from the pituitary glands, ovaries, and uteri were processed for prparation of 5 µm thickness histological sections, stained with hematoxylin and eosin stains, and examined by light microscopy according to Edna and Lestie [14].

Gene expression analysis: Using the TRIzol® reagent kit, total RNA was extracted from ovarian tissue samples (as instructed by Bioneer Co., South Korea). A nanodrop spectrophotometer was used to determine the amount and purity of RNA, as stated by Promega Company, USA. The trace amount of DNA was eliminated using DNase I (Promega Company, USA). Next, cDNA was synthesized using the AccuPower® RockScript RT PreMix Kit (Bioneer Co., South Korea). Using AccuPowerTM, a quantitative RT-PCR master mix was finished, and the amplification of the genes under study was determined using SYBER Green dye (Bioneer Co., Korea). The relative quantitative gene expression levels (fold change) of the target and housekeeping gene qRT-PCR findings were used for analysis [15].

Ethical approval:

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

Statistical Analysis: All data were presented as mean ± standard deviation. GraphPad Prism Version 5 (SAS Institute, Inc., USA) was used for statistical analysis of the results, using one way analysis of variance (ANOVA I) and Newman-Keuls test [16] to denote the significant differences between the means. $P<0.05$ is stated as significant.

Results

Genital organ weight

As illustrated in table (1), left and right ovarian weight at the estrus phase was significantly higher ($p<0.05$) among other estrous phases, followed by the proestrus, diestrus and anestrus phases, respectively. As for the weight of the uteri, it was significantly ($p<0.05$) higher at the diestrus phase compared with other estrous phases, which did not show any significant differences ($p>0.05$) between each other.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

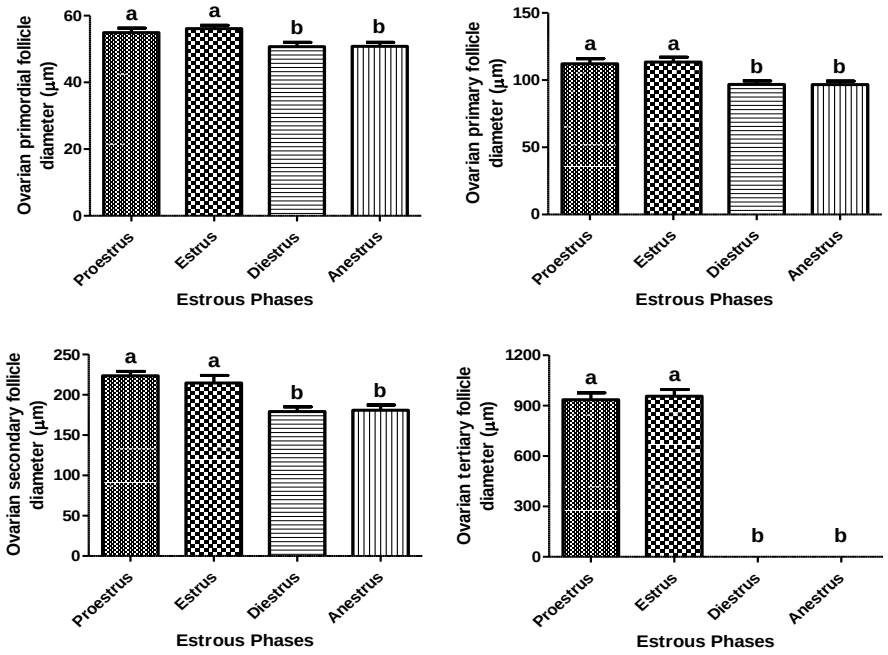
Table (1): Genital organ weight of adult queens in the reproductive phases

Organ Wt. (g/100g. bw)	Reproductive Phases			
	Proestrus	Estrus	Diestrus	Anestrus
Left ovary	0.0128±0.00014 **b**	0.0133±0.00035 **a**	0.0117±0.00027 **c**	0.0107±0.00030 **d**
Right ovary	0.0125±0.00017 **b**	0.0127±0.00026 **a**	0.0115±0.00025 **c**	0.0106±0.00023 **d**
Uterus	0.0809±0.0006 **b**	0.0827±0.0007 **b**	0.5472±0.2901 **a**	0.0548±0.0009 **b**

The values denote M ± SD. The different small letters denote significant differences ($p<0.05$) among estrous phases.

Ovarian follicle diameter: The ovarian primordial, primary, secondary, and tertiary follicles during proestrus and estrous phases recorded the higher diameter (µm) ($p<0.05$) compared with other estrous phases, whereas the diameter during diestrus and anestrus phases showed no significant differences ($p>0.05$) (Figure-1).

4.4.2. Ovarian corpus albicans, corpus luteum, and atresia follicle: As illuatrated in figure (2), the results showed insignificant differences ($p>0.05$) in the ovarian corpus albicans diameter (µm) between the four phases of the estrous cycle. The diameter of ovarian corpus luteum (µm) during diestrus phase was significantly higher ($p<0.05$) than that of other estrous phases, while that of proestrus and estrus phase was significantly higher ($p<0.05$) than anestrus phase. The ovarian atresia follicle during proestrus and estrous phases recorded decreased diameter (µm) ($p<0.05$) among other estrous phases, whereas the diameter during diestrus and anestrus phases showed no significant differences ($p>0.05$) (Figure-2).



Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Figure (1): Ovarian primordial, primary, secondary, and tertiary follicle diameter in different estrous phases of queens.
Different small letters denote significant differences (p<0.05) between the phases.

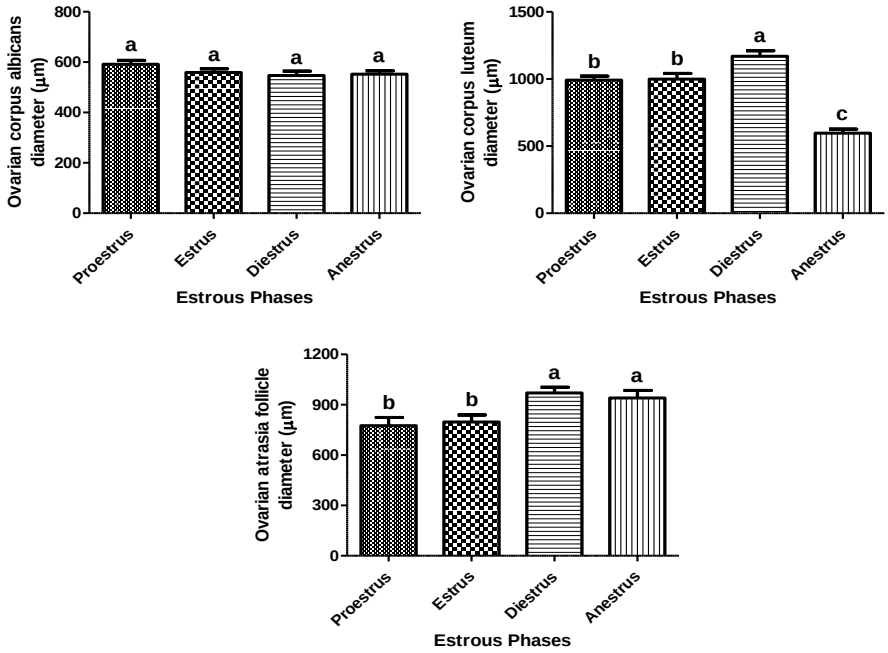


Figure (2): Ovarian corpus albicans, corpus luteum, and atrasia follicle diameter in different estrous phases of queens.
Different small letters denote significant differences (p<0.05) between the phases.

Myometrium, myometrium basalis, and myometrium functionalis: As shown in figure (3), the thickness of the myometrium (µm) during the proestrus phase was significantly higher (p<0.05) than that of other estrous phases, while that of the estrus and diestrus phases was significantly higher (p<0.05) than anestrus phase. The myometrium basalis thickness (µm) during estrus phase recorded the higher diameter (p<0.05) among other estrous phases, whereas the diameter during proestrus, diestrus, and anestrus phases showed no significant differences (p>0.05) (Figure-3). The thickness of myometrium functionals (µm) during proestrus was significantly higher (p<0.05) than that of other estrous phases, while that of estrus phase was significantly higher (p<0.05) than diestrus and anestrus phases which showed no significant (p>0.05) difference between each other.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

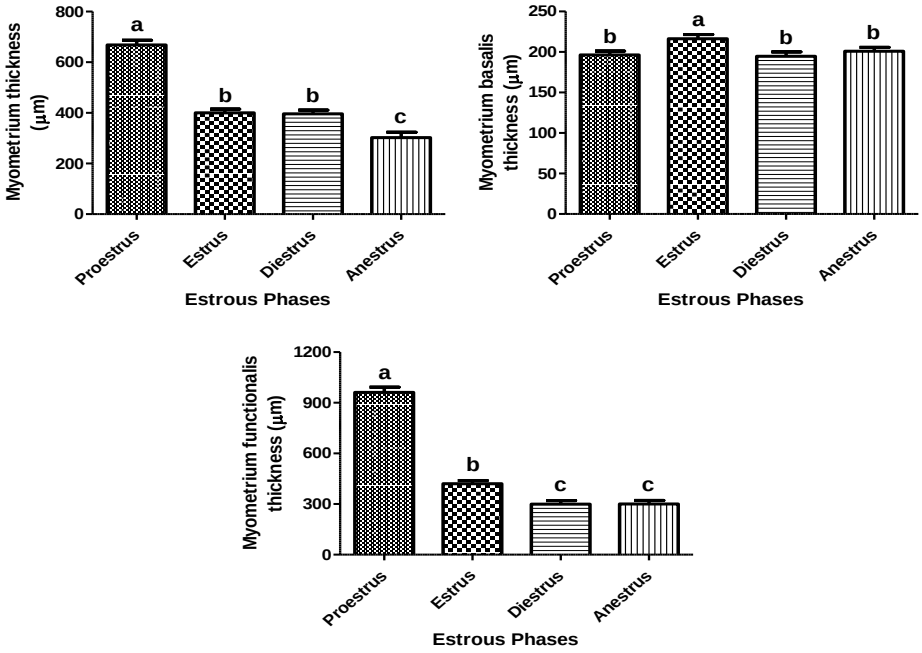


Figure (3): Myometrium, endometrial basalis, and endometrial functionalis layer thickness in different estrous phases of queens.
Different small letters denote significant differences ($p<0.05$) between the phases.

Pituitary gland, pituitary acidophils, and pituitary basophils: The diameter of the pituitary gland (µm) during proestrus, estrous, diestrus and anestrus phases showed no significant differences ($p>0.05$) (Figure-4). The pituitary acidophils during proestrus, estrous, diestrus, and anestrus phases showed insignificant differences ($p>0.05$) in their diameter (µm). The pituitary basophils diameter (µm) during proestrus phase recorded decreased diameter ($p<0.05$) among other estrous phases, whereas the diameter during estrus, diestrus, and anestrus phases showed no significant differences ($p>0.05$) between each other (Figure-4).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

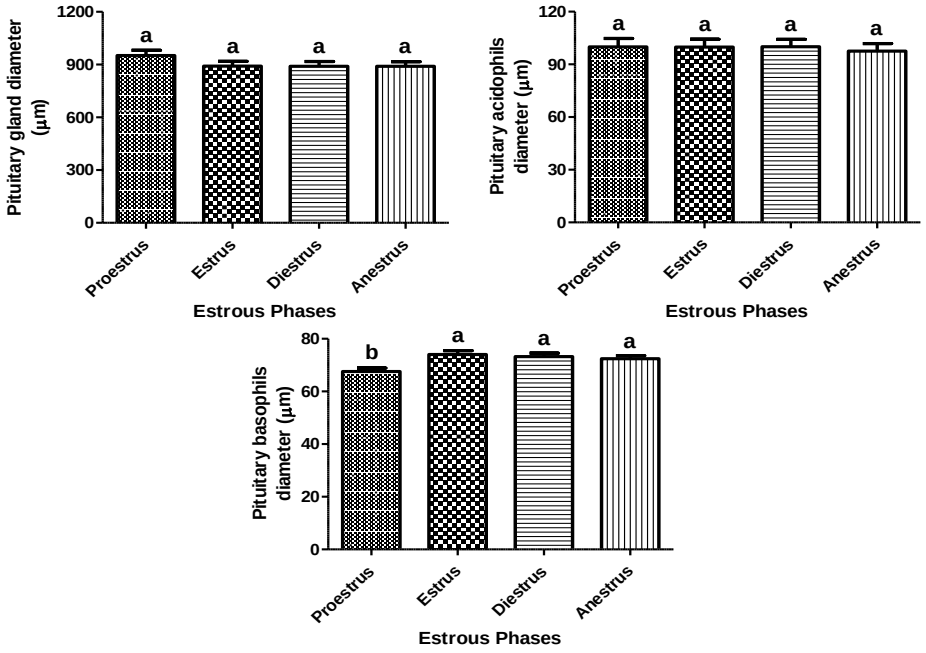


Figure (4): Pituitary gland and their acidophils and basophils diameter and its epithelium height in different estrous phases of queens.
Different small letters denote significant differences ($p<0.05$) between the phases.

Histophysiological findings of the ovary: The ovarian sections obtained from adult queens at the proestrus phase (Figure-5A), shows the cubic germinative epithelial cells from the outside encircled the ovarian tissue of the cats. A layer of tunica connectiva is also present. On the surface of the germinative epithelium, albuginea was seen. Follicles at various phases of development are found in the cortical layer. Also, the different stages of follicles in the ovarian cortex was shown which reflected by increased ovarian growth. Corpus luteum and sub-germinal tunica albuginea were also found. At the estrus phase, the ovary's sections (Figure-5B) shows that the tertiary follicles fall apart and granulosa cells, theca interna cells grow bigger and move into the cavity to form the granulosa lutein. The ovarian findings at the diestrus phase (Figure-5C) reveals the creation of corpus luteum and atresia follicles in the ovarian cortex. At the anestrus phase, the ovarian sections (Figure-5D) reveals primordial follicles, primary follicles, secondary follicles, corpus luteum, corpus albcains, and sub-germinal tunica albuginea.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

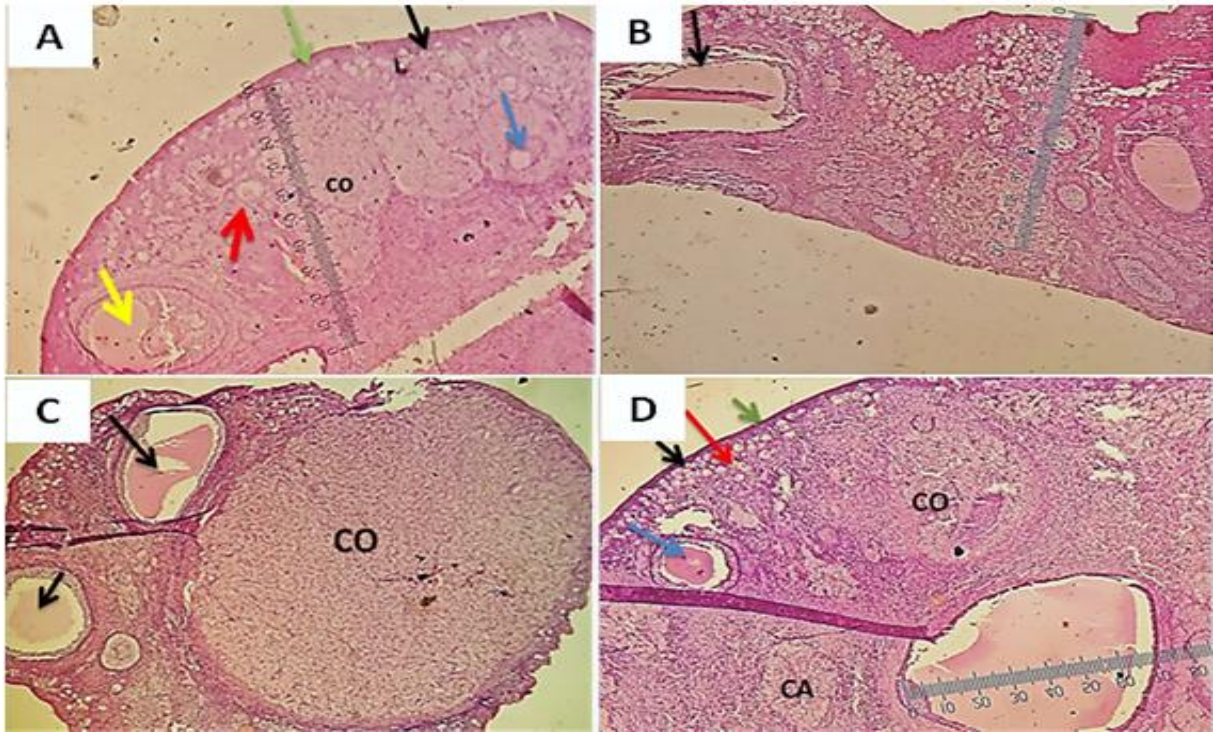


Figure (5): Histological section of ovary in cats in different estrous phases. The proestrus (A) showes different stage of follicles in the ovarian cortex. Small follicles or primordial follicles (black arrows), primary follicles (red arrows), secondary follicles (blue arrows), tertiary follicle (yellow arrow), corpus luteum (CO), and sub-germinal tunica albuginea (green arrows). estrus (B) phases show tertiery follicles (black arrows) collapses and the granulosa cells together with those of the theca interna, hypertrophy expanding in to the cavity to form the granulosa lutein and theca lutein cells of the corpus luteum in the ovarian cortex. Diestrus (C) show formation of the corpus luteum (CO), atresia follicels (black arrows) in the ovarian cortex. anestrus (D) phases show small follicles or primordial follicles (black arrow), primary follicles (red arrow), secondary follicles (blue arrow), corpus luteum (CO), corpus albcains (CA), and sub-germinal tunica albuginea. H and E stains: X40.

Histophysiological findings of the uterus: The histological sections taken from the uterus of a adult female cat during the proestrus phase (Figure-6A) reveals uterine glands, where some of them appeared as striated glands and others appeared lengthening glands. The sections also showed increased vascularization and congesting as well as the presence of oedam in the fuctionalis and basalis layers. In the histological sections of a adult female cat's uterus, collected during the estrus phase (Figure-6B), the uterine glands appeared as elongated glands, as well as the enhanced vascularization and congesting, while a diffused oedam present in the functionalis and basalis layers. The histological sections collected from the uterus of adult queens, taken during the diestrus phase (Figure-6C), shows increased uterine glandular development with reduced vascularization and congesting in the fuctionalis and basalis layers, as well as the presence of embryo implantation. The histological sections of adult female cat's uteri, collected during the anestrus phase (Figure-6D), shows fewer developmental changes in the uterine glands, where they illustrated normal inner and outer myometrial circular and

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

longitudinal layers, normal vascular stratum, and normal perimterum .

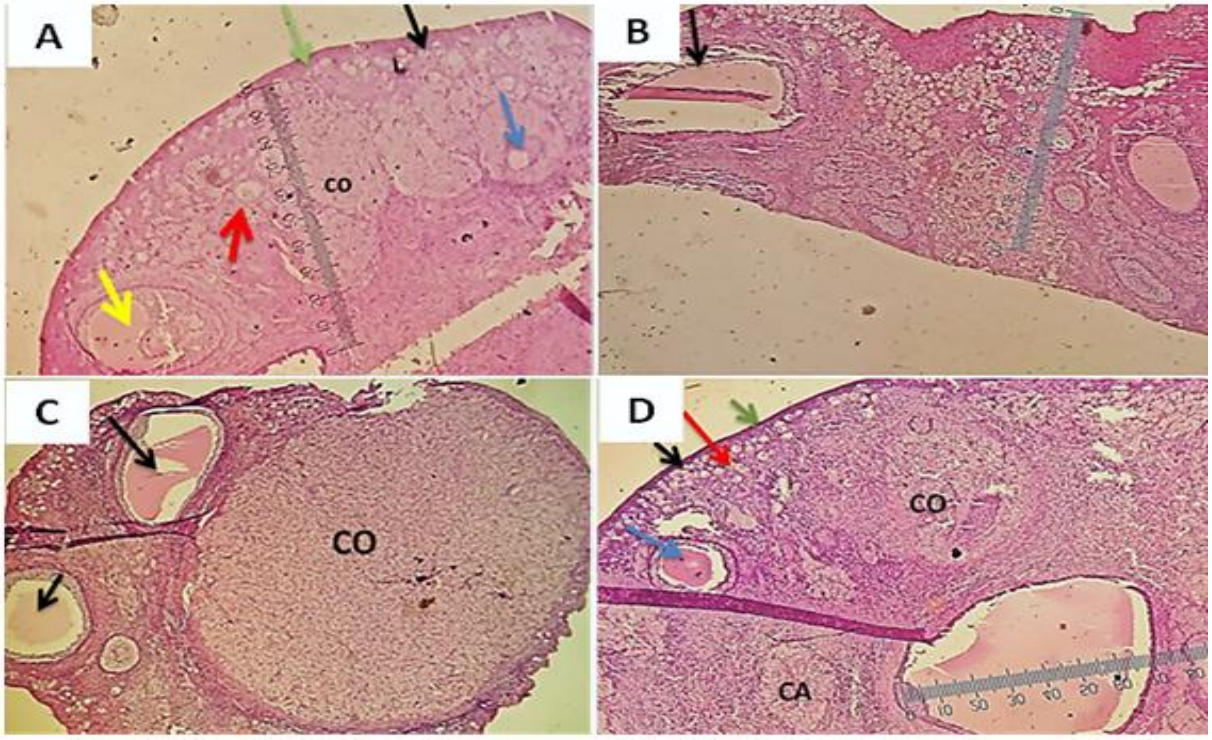


Figure (6): Histological section of uterus in cats in different estrous phases. The proestrus (A) and estrus (B) phases show the uterine glands (yellow arrow) appear as elongated glands, increased vascularization and congesting as well as difused oedam in the fuctionalis and basalis layers. Diestrus (C) phases show high uterine glandular development (black arrow), less vascularization and congesting in the fuctionalis and basalis layers, embryo implantation anestrus (D) phases show fewer developmental change in the uterine glands (G), normal inner myometrial circular layer (MI) and outer myometrial longitudinal layer (MO), normal vascular stratum (blue arrow), and normal perimterum (black arrow). H and E stains: X40.

Histophysiological findings of the Pituitary gland: The histological sections taken from the pituitary glands of adult queens during the proestrus phase (Figure-7A) and the estrus phase (Figure-7B), reveals a reflecting of the typing of hormones found in the cells, as a discrepancy staining characteristics and the size of secretion granules differs among the dissimilar types of the cells (acidophils and basophils). pituitary gland diameter and pituitary acidophils diameter appeared normal in size, whereas the pituitary basophils diameter decreased. The histological sections taken from the pituitary glands of adult queens during the diestrus phase (Figure-7C) and anestrus phase (Figure-7D), reveal differential staining that reflects the different types of hormonal content of the cells. as a discrepancy staining characteristics and the size of secretion granules differs among the dissimilar types of the cells between the acidophils and basophils. Furthermore, the pituitary gland diameter, pituitary acidophils diameter, and pituitary basophils diameter appeared normal in size.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

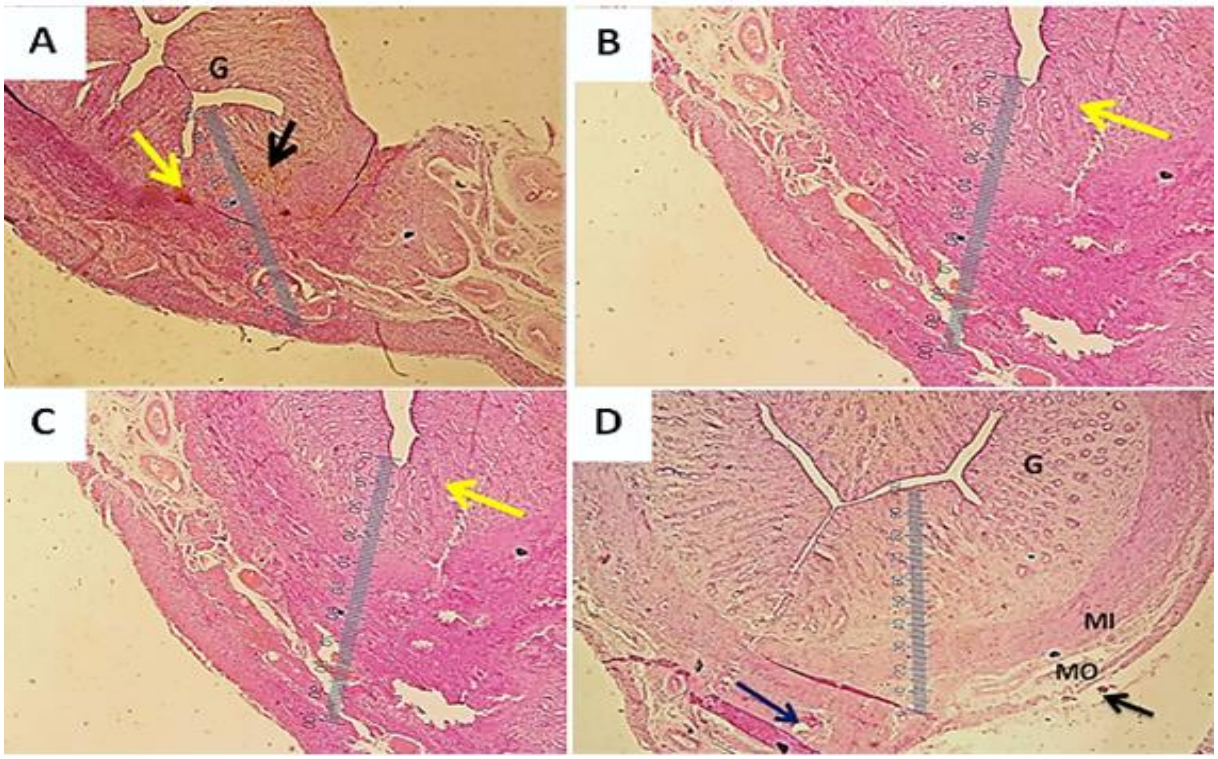


Figure (7): Histological section of pituitary glands in cats in different estrous phases. The proestrus (A) , estrus (B), Diestrus (C) and anestrus (D) phases show the different staining patterns seen are caused by the different hormones in the cells. are different from the way Acidophils (CM) and Basophils (SC) cells are spread out in equal amounts. H and E stains: X400.

Ovarian *fshr* and *lhr* gene expression: Figures (8) demonstrate that the fold changes of ovarian *fshr* and *lhr* genes were significantly ($p<0.05$) increased in estrus and proestrus phases compared with diestrus and anestrus phases, while proestrus and estrus phases and diestrus and anestrus phases showed insignificant differences ($p>0.05$).

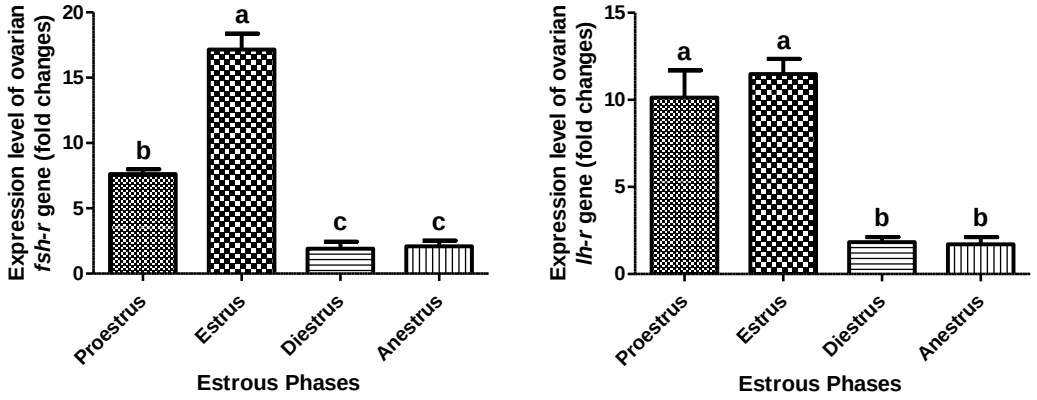


Figure (8): shows the fold changes of the ovarian *fshr* and *lhr* genes of female cats during various estrous phases.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Different letters indicate significant differences (p<0.05) between the ovarian phases.

Ovarian *inha* and cyp19a gene expression: Figures (9) demonstrate that the fold changes of ovarian *inha* and cyp19a genes were significantly (p<0.05) increased in proestrus and estrus phases compared with diestrus and anestrus phases, while proestrus and estrus phases and diestrus and anestrus phases showed insignificant differences (p>0.05).

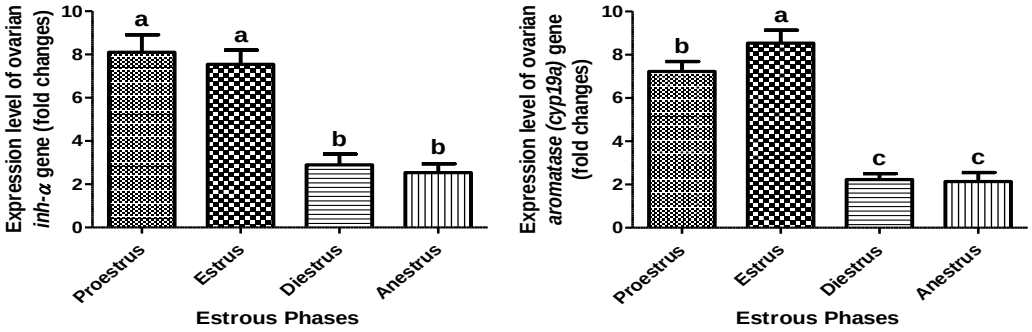
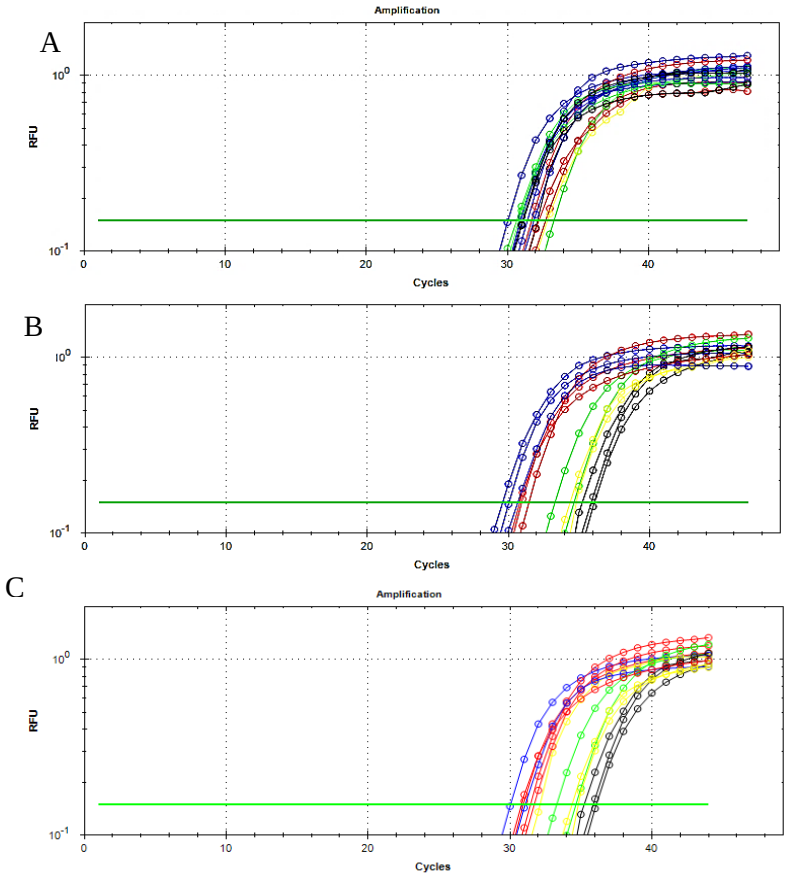


Figure (9): shows the fold changes of the ovarian *inhα* and aromatase (*cyp19a*) genes of female cats during various estrous phases.

Different letters indicate significant differences (p<0.05) between the ovarian phases.



Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

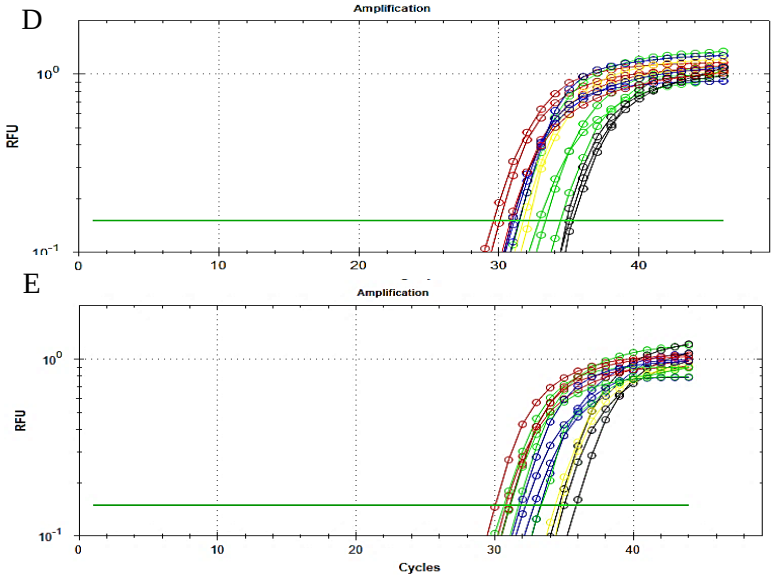


Figure (10): The real time PCR amplification plots of housekeeping (A), *fshr* (B), *lhr* (C), *inh-α* (D) and aromatase (*cyp19a*) (E) genes in the cat ovarian tissue. Estrus phase (blue), proestrus phase (red), anestrus phase (green), diestrus phase (yellow), and interestrus phase (black).

Discussion

Relative genital organ weight

The intensification in the weight of the ovaries during the proestrus and estrus phases indicates an increase in ovarian activity during these phases, as the follicular growth and the increase in the secretory activity of follicular cells begin in the prostrus phase and reach their highest levels in the estrus phase [7]. This activity is due to the action of pituitary gonadotropins, as the secretion of pituitary FSH and LH increases significantly in these phases as a result of increased secretion of hypothalamic GnRH [17]. Also, an increase in FSH will lead to an increase in the ovarian folliculogenesis to prepare the required number of oocytes for ovulation, and as a result, the size and weight of the ovary increases in those two stages. In the diestrus and anestrus phases, the levels of FSH and LH are low due to the low level of hypothalamic GnRH, and accordingly, the follicular growth is low during these two phases, so we found that the weights of the ovaries are low compared to the estrus and pre-estrus phases.The significant increase in uterine weights during the diestrus phase is due to the presence of embryos and embryonic fluids, in addition to the increase in the thickening of the uterine wall layers as a result of pregnancy. As for the other reproductive phases, the results showed that the uterine weights during the estrus and proestrus phases tended to rise, but they did not reach the significant degree compared to the anestrus phase. Despite the insignificance of the increase, it indicates an increase in the thickness of the uterine wall and the accumulation of small amounts of fluid inside the uterine cavity as a result of the action of estrogen in parallel with the development of the ovarian follicules, as the estrogen actually stimulates the growth of the endometrium and the preparation of progesterone receptors [7]. Kenny and Woodruff [18] noted that estradiol stimulates the maturation and advancement of the endometrium and increases arterial blood flow because estrogen forms endometrial

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

tissue appropriate for insertion of fertilized ova and embryo development until the placenta and embryonic membranes, that also secrete progesterone and estrogen, develop.

Morphometric measurements and histological findings of ovaries and uteri

In this study, the queens in the proestrus and estrus phases showed higher ovarian and uterine weight compared to diestrus and anestrus phases, which may reflect the high level of ovarian folliculogenesis, as usual reproductive processes require normal proliferative processes of ovarian follicular cells [19], which it is obligatory for ovarian growth, and follicle proliferation progress until ovulation [20]. During proestrus and estrus phases, pituitary FSH output increases and stimulates the ovarian follicle growth and maturation. This may explain the substantial improvement of the relative weight of the ovaries and uteri. Since granulosa cell proliferation increased, estrogen secretion also increased [21].In agreement with previous studies conducted by Kong et al. [22] and López et al. [23], the present study demonstrated higher nuclear receptors of thyroid hormones presence in the ovarian tissues of queens in the proestrus and estrus phases, and therefore, thyroid hormones involved in the regulation of ovarian folliculogenesis and steroidogenesis via these receptors. Furthermore, the ovaries in our study displayed an increase in overall FSHR mRNA expression during the proestrus and estrus phases, which could be resulted from the increased proportions of follicles at these phases within the ovarian tissues, as in the cat, FSH binding has been demonstrated to increase with follicles [24].

Molecular analysis of ovarian *fshr*, *lhr*, *inhα*, *cyp19a* genes

Pituitary gonadotropins, metabolic hormones and local growth factors coordinate ovarian proliferation, oogenesis, and steroidogenesis [25,26], therefore quantitation the expression levels of ovarian receptor genes for gonadotrpins as well as *inhα* and aromatase genes is more reliable for evaluation the ovarian activity in different phases of the estrous cycle. The current study resulted in significant changes of queen's reproductive hormones in the different estrous phases. Among these changes, the proestrus and estrus phases revealed increased expression levels of the ovarian *fshr* and *lhr* genes in comparison with anestrus and diestrus phases.The up-regulation of ovarian *fshr* and *lhr* positively affect the growth and development of the ovaries, as well as an increase of ovarian functions including oogenesis, folliculogenesis, and manufucturing of estrogen and progesterone, which have important regulatory roles in the estrous cycle [27]. These results could be a result of the stimulatory effect of thyroid hormones by enhancement of FSH to stimulate aromatase activity in granulosa cells [28], where the current findings mentioned to increased expression levels of aromatase enzyme (*cyp19a* gene), which therefore an indication of stimulation of estrogen synthesis and ovulation, as thyroid hormones contribute in the regulation of the ovarian growth, development, and metabolic activities by amplifying the effect of gonadotropin on granulosa cells [29,30]. Increased FSH levels from the pituitary gland can stimulate ovarian proliferation, prevent follicular atresia, and induce the synthesis of LH receptor and cytochrome P-450 aromatase in granulosa cells, all of which may explain the recent uptick in fshr expression in ovarian follicles [22,31]. It has been noted that the expression of FSH and LH receptors in granulosa cells significantly increases in correspondence with follicular diameter and the level of follicular growth, which has been noted in the present study during proestrus and estrus phases, as the increased expression levels of ovarian *inhα* and aromatase (*cyp19a*) genes during the proestrus

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

and estrus phases coincided with the increase in the growth and development of ovarian follicles. The folliculogenic degree depends on the proliferation of granulosa cells and theca cells, which are responsible for inhibins and estradiol secretion, respectively [32]. Since FSH is the primary stimulant of ovarian folliculogenesis [33] and it is responsible for a wide range of female reproductive processes, including ovarian granulosa proliferation and differentiation, gamete synthesis, and inhibin and estrogen secretion [34,35], therefor, the high production of FSH and estradiol during the proestrus and estrus phases [33] could be the account for the higher levels of *inha* and *cyp19a* gene expression in the ovaries, as compared to the diestrus and anestrus phases.

Conclusion:

In conclusion, there are histomorphological and functional alterations of pituitary glands, ovaries, and uteri at the different estrous phases of adult queens, which are correlates with the degree of ovarian development and activities in the secretion of estradiol and the density of FSH and LH receptors.

Conflict of Interest: there is no conflict of interest

References

1. Seigal M. The cornell book of cats: A Comprehensive Medical Reference for Every Cat and Kitten. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1-58.
2. Feldman EC, Nelson RW. Feline reproduction. In: Kersey R, LeMelledo D, editors. Canine and Feline Endocrinology and Reproduction. 3rd ed. Missouri: Saunders; 2004. p. 1016-1043.
3. Brown JL. Female reproductive cycles of wild female felids. Anim Reprod Sci. 2011 Apr;124(3-4):155-62. https://doi.org/10.1016/j.anireprosci.2010.08.024
4. Shille VM, Lundstrom KE, Stabenfeldt GH. Follicular function in the domestic cat as determined by estradiol-17b concentrations in plasma: Relation to estrous behavior and cornification of exfoliated vaginal epithelium. Biol Reprod. 1979;21:953-963.https://doi.org/10.1095/biolreprod21.4.953
5. Brown JL. Comparative endocrinology of domestic and nondomestic felids. Theriogenology. 2006;66:25-36.https://doi.org/10.1016/j.theriogenology.2006.03.011
6. Kutzler MA. Estrus induction and synchronization in canids and felids. Theriogenology. 2007;68:354-374.https://doi.org/10.1016/j.theriogenology.2007.04.014
7. Brown JL, Comizzoli P. Female Cat Reproduction. In: Skinner MK, editor. Encyclopedia of Reproduction. Vol. 2. Academic Press: Elsevier; 2018. p. 692-701.https://doi.org/10.1016/B978-0-12-809633-8.20638-9
8. Simpson GM, England GC, Harvey M, editors. Manual of Small Animal Reproduction and Neonatology. Cheltenham, UK: British Small Animal Veterinary Association Pub; 1998. p. 11-16.
9. Johnston SD, Root-Kustritz MV, Olson PN. Canine and Feline Theriogenology. Philadelphia: WB Saunders; 2001. p. 389-474.
10. Griffin B. Prolific Cats: The Estrous Cycle. Scott-Ritchey Research Center. Vol. 23, No. 12 December 2001.
11. Feldman EC, Nelson RW. Canine and Feline Endocrinology and Reproduction. 3rd ed. Philadelphia: WB Saunders Co; 1996. p. 1016-1043.
12. Long S. Genetics and Reproduction Physiology of Dog and Cats. Philadelphia: WB Saunders Co; 2006. p.81-86.
13. Noaks DE, Parkinson TJ, England GCW. Arthur's Veterinary Reproduction and Obstetrics. Philadelphia: WB Saunders Co; 2003. p.37-40.
14. Edna B, Lestie H. Laboratory methods in histotechnology. Washington, D.C.: American Registry of Pathology; 1991.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-410.https://doi.org/10.1006/meth.2001.1262
16. Shiefler WC. Statistics for Biological Sciences. 2nd ed. Addison: Wesley Publ. Co.; 1980.
17. Petersen A. Reproductive Physiology of the Female Cat. Swedish University of Agricultural Sciences; 2015.
18. Kenny HA, Woodruff TK. Follicle size class contributed to distinct secretion patterns of inhibin isoforms during the rat estrus cycle. J Endocrinol. 2005;10:1210-1242.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

19. Stouffer RL, Xu F, Duffy DM. Molecular control of ovulation and luteinization in the primate follicle. Front Biosci. 2007;12:297-307.https://doi.org/10.2741/2065
20. Yin M, Wang X, Yao G, Lü M, Liang M, Sun Y, et al. Transactivation of microRNA-320 by microRNA-383 regulates granulosa cell functions by targeting E2F1 and SF-1 proteins. J Biol Chem. 2014;289:18239-57.https://doi.org/10.1074/jbc.M113.546044
21. Al-Saaidi JAA, Al-Jayashi GSM. Adenohypophyseal immunohistochemical expression levels of FSHβ in cyclic virgin female rats treated with steroid free bovine follicular fluid antiserum. OIIRJ. 2016;6:18-31.
22. Kong L, Wei Q, Fedail JS, Shi F, Nagaoka K, Watanabe G. Effects of thyroid hormones on the antioxidative status in the uterus of young adult rats. J Reprod Dev. 2015;61:219-27.https://doi.org/10.1262/jrd.2014-129
23. López Navarro E, Ortega FJ, Francisco-Busquets E, Sabater-Masdeu M, Álvarez-Castaño E, Ricart W, et al. Thyroid hormone receptors are differentially expressed in granulosa and cervical cells of infertile women. Thyroid. 2016;26:466-73.https://doi.org/10.1089/thy.2015.0416
24. Saint-Dizier M, Malandain E, Thoumire S, Remy B, Chastant Maillard S. Expression of follicle stimulating hormone and luteinizing hormone receptors during follicular growth in the domestic cat ovary. Mol Reprod Dev. 2007;74:989-996.https://doi.org/10.1002/mrd.20676
25. Craig J, Orisaka M, Wang H, Orisaka S, Thompson W, Zhu C, Kotsuji F, Tsang BK. Gonadotropin and intra-ovarian signals regulating follicle development and atresia: the delicate balance between life and death. Front. Biosci. 2007;12:3628-3639.https://doi.org/10.2741/2339
26. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012;10(1):49.https://doi.org/10.1186/1477-7827-10-49
27. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod. 1994;9:188-191.https://doi.org/10.1093/oxfordjournals.humrep.a138480
28. Sirard MA, Desrosier S, Assidi M. In vivo and in vitro effects of FSH on oocyte maturation and developmental competence. Theriogenology. 2007;68:S71-S76.https://doi.org/10.1016/j.theriogenology.2007.05.053
29. Zhang SS, Carrillo AI, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. Mol Hum Reprod. 1997;3:555-62.https://doi.org/10.1093/molehr/3.7.555
30. Sato E, Jiang JY. Follicular development and ovulation in hypothyroid rdw rats. Ital J Anat Embryol. 2001;106(2 suppl 2):249-56.
31. Tamura K, Hatsuta M, Watanabe G, Taya K, Kogo H. Inhibitory regulation of inhibin gene expression by thyroid hormone during ovarian development in immature rats. Biochem Biophys Res Commun. 1998;242:102-8.https://doi.org/10.1006/bbrc.1997.7919
32. Al-Sa'aidi JA, Baqir SM. Effect of passive immunization against inhibin-a subunit and non-steroid bovine follicular fluid (NSBFF) on mammary gland growth and development in primiparous female Wistar rats. Reprod Dom Anim. 2012;47(4):416-613.
33. Al-Saaidi JAA, Al-Charak AG. Effect of steroid-free follicular fluid antiserum on reproductive endocrine profile at estrous and metestrus phases in female rats. Iraqi J Vet Sci. 2020b;34(2):273-278.https://doi.org/10.33899/ijvs.2019.125925.1187
34. Rozell TG, Okrainetz RJ. FSH: one hormone with multiple forms, or a family of multiple hormones. In: Chedrese ED, editor. Reproductive endocrinology, a molecular approach. Saskatoon: Springer Science and Business Media; 2009. p. 144-160.https://doi.org/10.1007/978-0-387-88186-7_14
35. Bhardwaj A, Nayan V, Parvati M, Gupta AK. Inhibin: A role for fecundity augmentation in farm animals. Asian J Anim Vet Adv. 2012;7:771-789.https://doi.org/10.3923/ajava.2012.771.789