Iraqi Journal of Veterinary Sciences, Vol. 37, Supplement III, 2023 (97-102)
Proceedings of the 7th (2nd International) Scientific Conference, College of Veterinary Medicine, University of Al-Qadisiyah

Iraqi Journal of Veterinary Sciences

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Seasonal morphometric and functional changes of thyroid gland and relationship with thr1 and thr2 genes expression in queen ovary

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Article information

Article history:
Received 17 December, 2022
Accepted 07 November, 2023
Available online 05 December, 2023

Keywords:
Queen
Estrous cycle
Reproduction
Thyroid gland
Ovary

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Abstract

This study aimed to determine the thyroid gland activity during different estrous phases in female cats. Forty mature female cats (ten in each estrus phase) were utilized. Blood samples were taken to measure the levels of thyroid hormones (T3 and T4). The thyroid gland was taken for histological examination. The ovary was dissected for molecular study. The results of the diestrus phase showed a significant increase in the height of follicular epithelium compared to the other phases. In contrast, the estrus phase significantly increased compared to the proestrus and anestrus phases. The follicular diameter was substantially more significant in the proestrus phase than in the other estrus phases, which showed no significant difference. Histological sections of the thyroid gland during the proestrus and estrus phases showed an increase in the follicular size and the number of lining cells, where the follicles appear filled with colloidal matter. Diestrus and anestrus phases showed normal-sized follicles with low colloid content. Serum levels of T3 and T4 were significantly increased during the proestrus and estrus phases compared to other phases. The molecular analysis, on the other way, showed a significant increase in the ovarian *thr1* and *thr2* gene expression levels during the proestrus and estrus phases compared to different phases. It is concluded that there is a relationship between the thyroid gland's activity and the ovaries' functional activity during the proestrus and estrus phases.

DOI: 10.33899/ijvs.2023.137322.2669,

Introduction

The TG is an important endocrine gland that is essential for life as it is the unique source of thyroid hormones (THs), as the thyroid glands, anterior pituitary gland, and hypothalamus are the components that make up the hypothalamic-pituitary-thyroid axis, which is an automated circuit. T4 works in concert with thyroid stimulating hormone (TSH), which is secreted through the anterior pituitary glands, and thyroid releasing hormone (TRH), which is produced through the hypothalamus; all act in synchrony, which is responsible for maintaining a healthy feedback system and homeostasis (1). Specific receptors allow THs to enter all body tissues. The primary role of THs is to control energy and metabolism, but they also control a wide range of physiological processes, including female reproduction and the female ovarian cycle (2). L-thyroxine and L-triiodothyronine regulate the development and metabolism of the ovary, uterus, and placenta directly through nuclear receptors and indirectly by interacting with a variety of growth factors and hormones, such as gonadotrophin-releasing hormone, estrogen, insulin-like growth factor, and prolactin, that are released by the hypothalamic-pituitary-gonadal axis. Therefore, THs have a crucial role in regulating the metabolism, growth, and development of female genital organs, including the ovaries, as well as its role in the functioning condition of female reproduction (3) and its importance in the formation of ovarian preantral and antral follicles (4).

Cats are kept as pets worldwide and play an essential role in scientific research in genetics, fertility, neurophysiology, developmental biology, oncology, and infectious disease (5). Female cats reach puberty between 5 and 12 months, and free-roaming queens undergo seasonal polyestrous cycles. A typical queen's season starts in February and finishes in late September. From October through December, anestrus lasts until the start of the new season. Some queens, especially those that reside in warm regions, may continue to cycle well into November before briefly going into anestrous. Proestrus, estrus, interests, diestrus, and anestrus are the five phases of the queen's estrous cycle, unique among domestic species. Queens have a wide variety of individual variances in cycle duration. Seasonally polyestrous, incredibly fertile, and capable of copulatory and non-copulatory stimulation, female cats can cause premature ovulation (6). Even in the middle of the breeding season, fewer days can cause anestrus to begin since the queen's estrous cycle depends on a photoperiod. A period of anestrus may start at higher temperatures, as is the case during the height of summer, as has also been hypothesized (7). As a result, it has been observed that the breeding season occasionally consists of two phases, one in the spring and one in the early fall, with an anestrus phase during the hotter summer months. Understanding the mechanisms of the estrous cycle, ovulation, and pregnancy has been critical in understanding reproductive physiology (8).

The current study aims to discover the morphological and endocrinological changes of the thyroid gland in the different stages of the estrous cycle in local female mature cats.

Materials and methods

Ethical approve

According to the ethics and policies guidelines of the University of Al-Qadisiya, the current study was carried out.

Experimental animals

Domestic mature female cats (aged between 1 and 3 years) were included in the current study. Feral cat traps captured female cats at different phases of the estrous cycle.

Experimental protocol

Forty queens dived according to the estrous cycle stage. Ten at each of the proestrus (January, the temperature ranged between 8-15 °C), estrus (February, the temperature ranged between 4-10 °C), diestrus (April to July, the temperature ranged between 18-38 °C), and anestrus (March to October, the temperature ranged between 20 45 °C) phases, were captured, anesthetized with ketamine (90 mg/kg BW) and xylazine (40 mg/kg BW), and euthanized (the animals were handled according to the international and national animal welfare standards). The estrous phases were determined according to the morphological changes of ovaries and uteri after euthanization (5). Blood samples were obtained to assess T4 and T3 in the sera. The thyroid glands were dissected and fixed in 10% formalin for the histological examination. The ovaries were dissected and immediately stored in DEPC for molecular analysis.

Blood collection and sera preparation

After collection, the blood samples were centrifuged at 5000 rpm for 10 minutes, and sera were aspirated into Eppendorf tubes and kept at -20°C until hormonal assays (9).

Hormonal assay

Using the ELISA technique, cat kits were used to assess the serum concentrations of T3 and T4 (nmole/L) according to the manufacturer's instructions (Ltd. Com, China, and Shanghai Biological Co., Ltd., China).

Preparation, staining, and examination of the histological sections

The dissected specimens from the thyroid glands were immediately transported to a 10% formalin solution. The histological sections (5 µm thick) were prepared, stained with hematoxylin and eosin stains, and examined by light microscopy according to Edna and Lestie (10).

Molecular analysis (gene expression)

Total RNA from ovarian samples was extracted using the TRIzol® reagent kit (according to the instructions of Bioneer Co., South Korea). As described by Promega Company, USA, the quantity and purity of RNA were estimated using sing a Yield Nanodrop spectrophotometer, and DNase I Enzyme was used to remove the trace amount of DNA (Promega Company, USA). The AccuPower® RockScript RT PreMix Kit (Bioneer Co., South Korea) was then used to synthesize cDNA. A quantitative RT-PCR master mix was completed using AccuPowerTM and depended upon SYBER Green dye to determine the amplification of the studied genes (Bioneer Co., Korea). The qRT-PCR results of target and housekeeping genes were analyzed using the relative quantitative gene expression levels (fold change) ((ΔCT method using reference genes) described by Livak and Schmittgen (11).

Statistical analysis

Data were expressed as mean ± standard deviation. The results were statistically analyzed using GraphPad Prism Version 5 (SAS Institute, Inc., USA). Newman-Keuls (12) and one-way ANOVA were used to determine the significant differences between the means. $P \leq 0.05$ is regarded as substantial.

Results

Height of thyroid follicle epithelium

As illustrated in figure 1, the size of thyroid follicle epithelium (µm) during diestrus was significantly higher

(P<0.05) than that of other estrous phases, while that of the estrus phase was significantly higher (P<0.05) than proestrus and anestrus phases, which showed no significant (P>0.05) difference between each other.

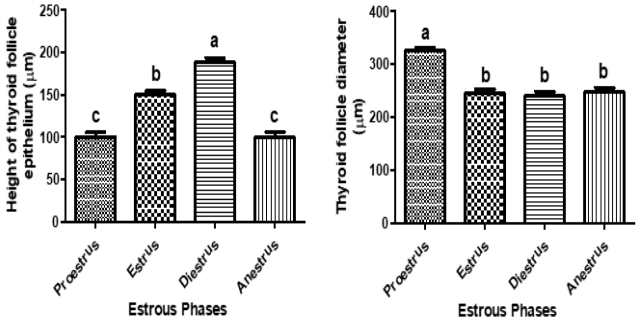

Figure 1: Thyroid follicle epithelium height and diameter in different estrous phases of female cats. Data were presented as Mean ±SD. Different small letters denote significant differences (P<0.05) between the phases.

Histophysiological findings of the thyroid gland

Histological sections taken from the thyroid gland of a mature female cat during the proestrus and estrus phases reveal increased follicular size and epithelial cells that lined the follicles, with the epithelial cells appearing as simple cuboidal or tall columnar, and the follicles filled with colloid materials. On the other hand, the histological sections of thyroid glands obtained in diestrus and anestrus phases revealed normal architecture with the normal size of the follicles, which are filled with a scanty amount of colloid. At the same time, the follicular lining cells are simple, tall, and cuboidal in shape (Figure 2).

Serum concentrations of T3 and T4

Serum concentrations of T4 and T3 (nmole/L) in the proestrus and estrus phases were significantly (P<0.05) higher than their concentrations in the diestrus and anestrus phases, whereas insignificant (P>0.05) differences were shown between the proestrus and estrus phases and also between the diestrus and anestrus phases (Figure 3).

Molecular analysis of ovarian *thr1* and *thr2* genes

As shown in figures 4 and 5, the fold changes of ovarian thr1 and thr2 genes were increased significantly (P<0.05) in proestrus and estrus phases compared with diestrus and anestrus phases, whereas insignificant (P>0.05) differences were shown between proestrus and estrus phases and also between diestrus and anestrus phases.

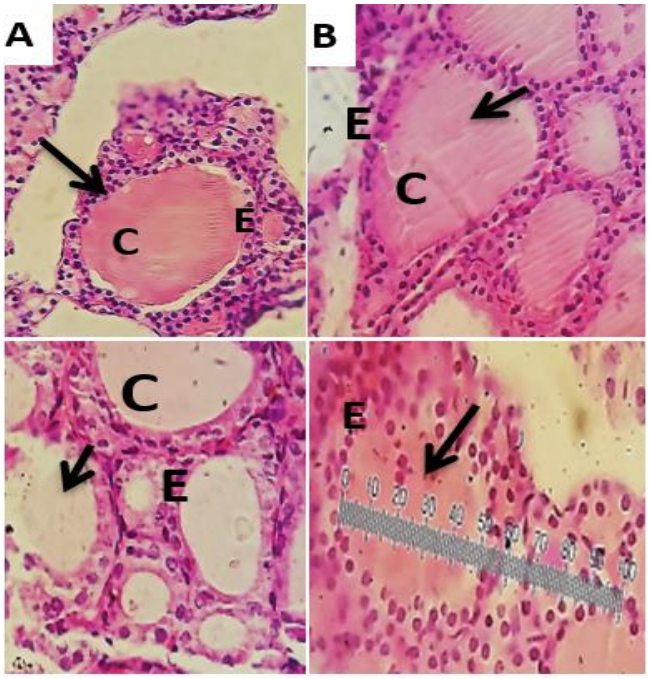

Figure 2: Histological section of thyroid glands in cats in different estrous phases. The proestrus (A) and estrus (B) phases show the increasing size of follicles (black arrows) and epithelium (E) during the activity phases. The epithelial cells appear as simple cuboidal or tall columnar structures, and the follicles are filled with colloid materials (C). Diestrus (C) and anestrus (D) phases show normal thyroid follicle size (black arrow), a scant amount of colloid (C), and simple tall cuboidal lining cells (E). H and E stains: X400.

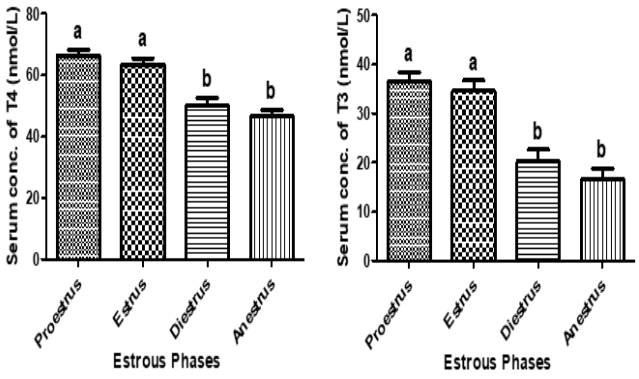

Figure 3: Serum concentrations of T4 and T3 in different estrous phases of the female cat. Data were presented as Mean ±SD. Different small letters denote significant differences (P<0.05) between the phases.

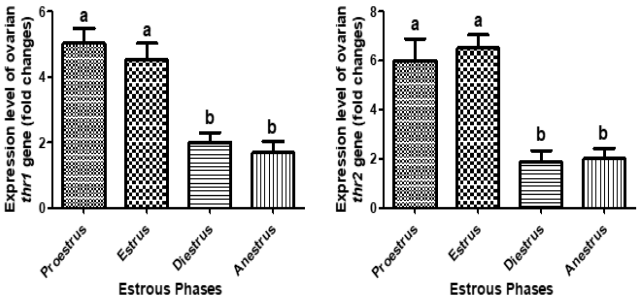

Figure 4: The expression levels (fold changes) of ovarian *thr1* and *thr2* genes in different estrous phases of a female cat's ovarian tissue. Data were presented as Mean ±SD. Different small letters denote significant differences (P<0.05) between the phases.

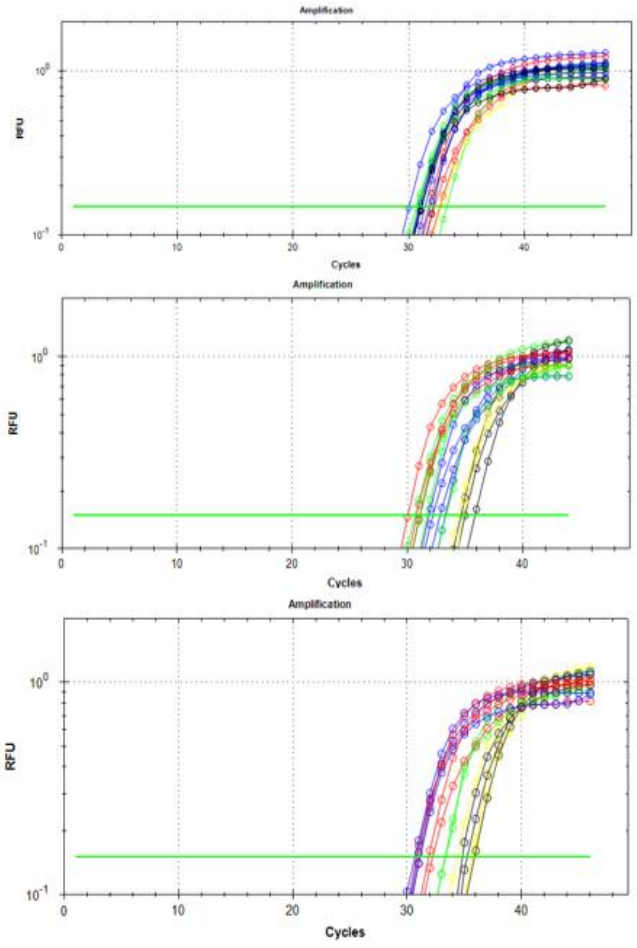

Figure 5: The real-time PCR amplification plots of housekeeping (A), *thr1* (B), and *thr2* (C) genes in the cat ovarian tissue. The blue plots (estrus phase), the red plots (proestrus phase), the green plots (anerestrus phase), the yellow plots (diestrus phase), and the black plots (interestrus phase).

Discussion

The decreased height of thyroid follicular epithelium and the increased diameter of the follicles during the proestrus phase could be due to the eventual accumulation of luminal colloid substances in the epithelial cells of the thyroid follicles, which in turn increases the intrafollicular pressure and increases the tension in the follicular wall, which may contribute to the flattening of follicular cells (13).

During the proestrus and estrus phases, the increased follicular size, epithelial cellular proliferation, and the accumulation of colloid materials indicate the advanced functional state in line with the entry of female cats into estrus, which requires the provision of energy and all the necessary needs for the ovarian proliferation in terms of follicular growth and development. Various studies have examined the relationship between thyroid state and ovarian functions (13,14). As in hypothyroidism, an impairment of functional ovarian reserve was noticed in mice and rats supplemented with low-iodine diets, as well as a decline in the number of primordial, primary, and preantral follicles. On the other hand, since the common histologic features of the thyroid gland, the brush cutter glands, were similar to those of other mammals (15,16), the follicular profiles in the present study were consistent with those recorded by Moskalenko *et al.* (12), as the current study showed that large follicles are usually peripheral and small ones were centrally located. Moreover, the presence of peripheral unevenness and vacuoles of follicles of different sizes in proestrus and estrus phases in the current study suggests higher thyroid activity.

Since thyroid hormones can be affected by various physiological and pathological circumstances, they are frequently tested to evaluate thyroid activity (17). T4 is the predominant hormone produced by the thyroid's follicular cells, and it has a longer half-life than T3, which has a shorter half-life and is more physiologically active (18). The HPT axis typically controls THs homeostasis because hypothalamic TRH governs pituitary TSH secretion, which controls thyroid THs secretion. On the other hand, the activities of T3 and T4 on the hypothalamus and anterior pituitary negatively influence this axis (19). Due to the favorable correlation between thyroid hormones and reproductive steroids (20), female cats' serum T4 and T3 levels are currently elevated during the proestrus and estrus phases. The HPT and HPG axes control reproductive processes (21). Different impacts of thyroid hormones (THs) are seen in the gonads (22), as THs play a role in the gonads' typical growth, development, and operations. It has been reported that ovarian tissues and cells can control the expression of iodothyronine deiodinases, an enzyme involved in TH metabolism, TH transporters, and TH receptors, depending on the life stage. Independent of changes in their circulating levels, the local regulation of

THs maintains the peripheral T3 and T4 levels necessary for ovarian activity (23,24).

According to the results of the current study, the ovarian *thr1* and *thr2* gene expression levels were considerably elevated during the proestrus and estrus phases, indicating that both the thyroid and the ovary were functionally more active. Deiodinases are a class of intracellular enzymes that control thyroid hormone function, including deiodinase II, which converts T4 to T3 in the brain and pituitary gland (25). Thyroid receptors (*thr1* and *thr2*) support the effects of thyroid hormones (26-28). Thyroid receptors are found in many bodily tissues, including ovarian follicular cells (29), and thyroid receptor downregulation lowers the number of follicles and affects fertility (30).

Conclusion

In conclusion, the functional activity of the ovaries during the proestrus and estrus phases is correlated with the thyroid gland's activity.

Acknowledgments

The authors would like to thank the deanery of the College of Veterinary Medicine, University of Al-Qadisiyah, for their support in performing this research.

Conflict of interest

No conflict of interest was found.

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التغيرات الشكلية والوظيفية الموسمية للغدة الدرقية والعلاقة مع تعبير جيني *thr1* و *thr2* في مبايض إناث القطط

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الخلاصة

أجريت هذه الدراسة لمعرفة نشاط الغدة الدرقية أثناء أطوار دورة الشبق المختلفة في إناث القطط. تضمنت الدراسة ٤٠ من إناث القطط الناضجة بواقع ١٠ إناث في كل طور من أطوار الشبق. تم أخذ عينات دم لغرض قياس مستوى هرمونات الدرقية الثيروكسين الثالث والثيروكسين الرابع، كما تم أخذ عينات من الغدد الدرقية لغرض الدراسة النسجية وتم أخذ عينات من المبايض لغرض الدراسة الجزيئية. أظهرت نتائج طور من الشبق زيادة معنوية في ارتفاع الخلايا البطانية للجريبات بالمقارنة مع الأطوار الأخرى بينما أظهر طور الشبق زيادة معنوية عن طوري قبل الشبق وخارج أو اللاشبق. كان قطر الجريبات في طور قبل الشبق الأعلى معنويا من بين أطوار الشبق التي أظهرت عدم وجود فرق معنوي فيما بينها. أظهرت المقاطع النسجية للغدد الدرقية أثناء طوري قبل الشبق والشبق زيادة في حجم الجريبات الفارزة وأعداد الخلايا المبطنة للجريبات إذ تظهر الجريبات ممتلئة بالمادة الغروية، بينما أظهرت المقاطع في طوري من الشبق واللاشبق جريبات طبيعية الحجم والمحتوى من المادة الغروية كان قليلا. ارتفعت مستويات الثيروكسين الثالث والثيروكسين الرابع معنويا أثناء طوري قبل الشبق والشبق بالمقارنة مع طوري من الشبق واللاشبق. من جانب آخر، أظهرت الدراسة الجزيئية ارتفاعا معنويا في تعبير جيني *thr1* و *thr2* في أنسجة المبايض خلال طوري قبل الشبق والشبق بالمقارنة مع طوري من الشبق واللاشبق. يستنتج من نتائج الدراسة الحالية وجود علاقة بين نشاط الغدة الدرقية النشاط الوظيفي للمبايض أثناء طوري قبل الشبق والشبق.