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Serum Triiodothyronine, Thyroxine, and Thyroid Stimulating Hormone Concentrations of Domestic Female Cats at Different Reproductive Stages

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Abstract: Thyroid hormones have various roles in different physiological systems in the body. Thus, thyroid dysfunction is common in cats, in this study, we aimed to determine the normal values of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) in the blood of female cats at different phases of the estrous cycle. Queens were divided into three groups as estrous (n=14), diestrus (n=12), and interestrus (n=10) according to findings of vaginal cytology, ovarian inspection, and serum estradiol (E2) and progesterone (P4) concentrations. Blood samples were collected before the ovariohysterectomy, and the obtained sera were analyzed for free T3, free T4, TSH, E2, and P4. The results showed that the highest T3 and T4 concentrations were found in cats at interestrus while the lowest T3 and T4 concentrations were found at diestrus and estrus, respectively. The level of TSH in all cats was below the detection limit of the assay. The mean concentrations of thyroid-related hormones in each group showed non-significant variations (P>0.05). The correlation between ovarian and thyroidal hormones was not statistically significant (P>0.05). In conclusion, it is required to further comprehensive/experimental studies to exhibit the interaction between ovarium and thyroid gland because of its importance.
Keywords*: Estrous stages, Feline, Reproductive cycle, Thyroid hormones, TSH.*

Farklı Üreme Dönemindeki Dişi Kedilerin Serum Triiyodotironin, Tiroksin ve Tiroit Uyarıcı Hormon Konsantrasyonları

Özet: Tiroit hormonları vücuttaki birçok fizyolojik sistemin gelişim ve fonksiyonlarına dahil olmaktadır. Kedilerde tiroit bozuklukları sık görüldüğünden, bu çalışmada östrus siklusunun farklı evrelerindeki dişi kedilerin kanında bulunan tiriyodotironin (T3), tiroksin (T4) ve tiroit uyarıcı hormonun (TSH) normal değerlerinin belirlemesi amaçlandı. Kediler vajinal sitoloji, ovaryum muayenesi ve serum östrojen (E2) ve progesteron (P4) konsantrasyonlarının bulgularına göre östrus (n=14), diöstrus (n=12) ve interestrus (n=10) olmak üzere üç gruba ayrıldı. Ovariohisterektomiden önce kan örnekleri toplandı ve elde edilen serumlardan E2 ve P4'ün yanı sıra serbest T3, serbest T4 ve TSH analizi yapıldı. Bu çalışmanın sonuçları, en yüksek T3 ve T4 konsantrasyonlarının interöstrusdaki kedilerde olduğunu, en düşük T3 ve T4 konsantrasyonlarının sırasıyla diöstrus ve östrusdaki kedilerde olduğunu gösterdi. Tüm kedilerdeki serum TSH seviyeleri ise test kitinin tespit sınırının altındaydı. Gruplardaki tiroitle ilgili hormonların ortalama konsantrasyonları anlamlı olmayan farklılıklar gösterdi (P> 0,05). Ayrıca ovaryum ve tiroit hormonları arasındaki korelasyon istatistiksel olarak anlamlı bulunmadı (P> 0,05). Sonuç olarak, önemi nedeniyle ovaryum ve tiroit bezi arasındaki etkileşimi ortaya koymak için daha kapsamlı/deneysel çalışmalara ihtiyaç olduğu düşünüldü.
Anahtar Kelimeler: *Kedi, Östrus siklusu, Tiroit hormonları, TSH, Üreme döngüsü*

Introduction

Triiodothyronine (T3) and thyroxine (T4) which also called thyroid hormones have a critical role in the development of the brain and body of a fetus and large number of metabolic functions in cardiovascular, nervous, immune, and reproductive systems of adults (Feldman et al., 2014). These hormones derived from tyrosine amino acid are mainly circulated by thyroid-binding proteins in the blood but, the small amount (less than 1%) of them are transported from the thyroid gland to target tissues as unbound, which are called free T3 and T4. Although the biologically active form of thyroid hormones is T3, there is less production of it in the thyroid gland compared to T4. Thyroxine is the major form of the thyroid hormones secreted by thyroid gland and in blood circulation. However, it is finally metabolized to T3 in target tissues/cells since it is synthesized as a prohormone of T3 (Barrett et al., 2016). The thyroid-stimulating hormone (TSH) is produced and secreted by the anterior pituitary gland to induce the thyroid gland for the biosynthesis of thyroid hormones. If blood concentrations of thyroid hormone are decreased, the pituitary gland is stimulated to release TSH, if thyroid hormone levels are increased, TSH secretion is suppressed. Therefore, it has also a quite importance in terms of the assessment of the activity of thyroid gland (Feldman et al., 2014).

Many variants can influence serum thyroid hormone concentrations such as species, breed, sex, age, and reproductive status like other biochemical parameters, or diseases unrelated to thyroid gland (Peterson et al., 1983; Yokus et al., 2006). Skinner (1998) showed that serum T3 and T4 levels in cats were gradually reduced depending on aging, while others remained within the normal ranges. The author also reported a gender-related difference in T4 decline, such that the age-related decrease in T4 was significant in females but not significant in males. In some previous studies, it has been shown that the physiological status of the ovary may lead to differences in blood thyroid hormone levels in cows (Ashkar et al., 2010; Soliman et al., 1963), buffaloes (Ghani et al., 2017), and bitches (Reimers et al., 1984).

Thyroid dysfunctions, especially hyperthyroidism, are commonly diagnosed diseases of the endocrine system in cats. However, the physiopathology of feline hyperthyroidism is still unclear. Therefore, veterinary practitioners and researchers are interested in this endocrinopathy (Peterson et al., 1987; Vaske et al., 2014). For the diagnosis of many diseases, it requires the comparison of clinical data obtained from potential patients with the reference values of healthy ones (Yokus et al., 2006). Thyroid and thyrotropic hormones are commonly measured to evaluate thyroidal activity. In some studies, thyroid-related hormones (total T3, total T4, free T3, free T4, and TSH) of cats have been assessed in different states such as in thyroidal (Peterson et al., 2015; Peterson et al., 1987) and non-thyroidal diseases (Mooney et al., 1996), and various environment and age (Skinner, 1998). It has been also reported that ovarian activity may affect the blood level of thyroid-related hormones in humans and animals (Ashkar et al., 2010; Krassas et al., 2010). However, to the best of our knowledge, no study on the variation of thyroidal activity during the estrous cycle has been reported in female cats.

The present study investigated the normal values of free T3, free T4, T4:T3 ratio and TSH levels of domestic female cats at different reproductive stages.

Materials and Methods

Animals: This study was conducted on 36 mix breed, mature (2.02 ± 1.30 years old) and healthy domestic female cats free from reproductive and metabolic diseases, which were brought to Small Animal Hospital of Kirikkale University/TURKEY for routine ovariohysterectomy. All cats which had no administration of any steroid or thyroid hormone treatments included to study and they underwent clinical examination and complete blood count. All of them were owned, fed with commercial dry food, and located in Kirikkale region. The whole procedures of this research were approved by the Local Ethical Committee of Kirikkale University, Turkey (2020/06-34).

Detection of estrous' stage: The estrous' stage was determined by findings of vaginal cytology that stained with Giemsa, serum estradiol and progesterone levels, and macroscopic examination of the existence of follicles or corpus luteum on ovaries as described previously (Kabakci et al., 2019; Mills et al., 1979). The percent distribution of three types of cells (parabasal, intermediate and superficial) were assessed in the vaginal cytology of cats. According to that, superficial (60-70%) and intermediate (40-60%) cells were in highest percentage during estrus and diestrus, respectively, while both were in average distribution (30-50%) during interestrus (Mills et al., 1979; Shille and Sojka, 1965).

Blood collection: Blood samples were collected into tubes with/without anticoagulant (EDTA) from the *vena cephalica antebrachia* of cats before each ovariohysterectomy at 10 am. Hematological analysis was performed, and cats have any metabolic diseases were not included to the study. The other blood samples allowed to clot at 4 ºC for approximately 20 min and then centrifuged at 1,000 g for 10 min. The separated sera were stored at -20ºC for analysis.

Hormone measurement: The E2 and P4 concentrations of sera were measured by using electrochemiluminescence immunoassay kits (Elecsys Estradiol III and Elecsys Progesterone III, Roche Diagnostic, USA) on the Roche Cobas E800 analyzer with the recommendations of the manufacturer. The findings of vaginal cytology and macroscopic examination were confirmed with the serum E2 and P4 concentrations. According to that, cats were classified as in estrous when E2 levels were higher than 20 pg/ml. The queens having higher P4 levels than 1.5 ng/ml were classified as in diestrus and, E2 and P4 levels lower than 20 pg/ml and 1.5 ng/ml respectively were classified as in interestrus (Hamouzova et al., 2017; Kabakci et al., 2020). The range limits of assays for E2 and P4 were 5 - 3000 pg/mL and 0.05 – 60 ng/mL, respectively.

The concentrations of free T3, T4, and TSH of the sera were also assessed by electrochemiluminescence immunoassay kits (FT3, Elecsys FT4 III, and Elecsys TSH, Roche Diagnostic, USA) on the Roche Cobas E800 analyzer with the recommendations of the manufacturer. The range limits of assays for free T3, free T4, and TSH were 0.4-50 pmol/L, 0.5-100 pmol/L, and 0.005-100 µIU/mL, respectively.

Statistical analysis: Before the significance analysis of the parametric data, normality test and homogeneity of variance were performed with Shapiro Wilk and Levene test, respectively. Statistical analysis of the data expressed the mean ± standard error mean (SEM) for each group was done by using one-way analysis of variance (ANOVA). The relationship between E2, P4, T3, T4, and T4:T3 (except TSH due to not detection) parameters was assessed by using the Pearson correlation coefficient. All statistical analysis was performed by using SPSS 14.01 package program and assessed 5% margin of error ($P<0.05$).

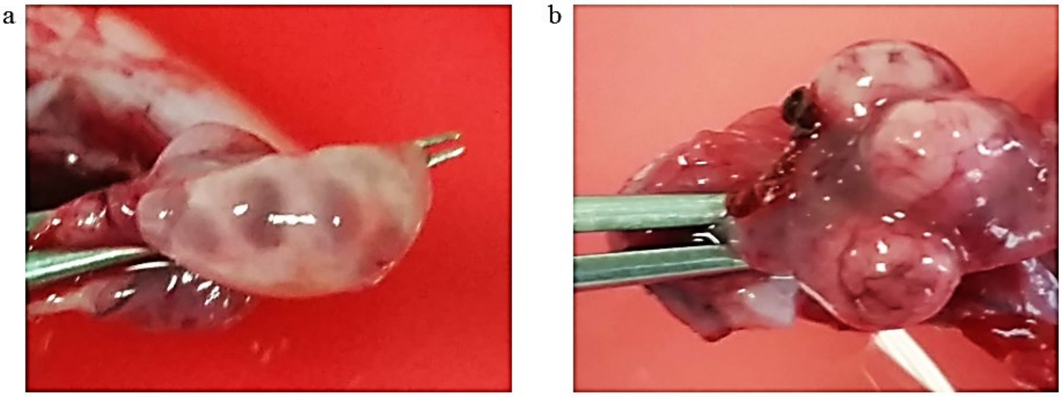

Figure 1. Macroscopic examination of feline ovaries during ovariohysterectomy. (a) Cats having one or more follicle on the ovary or ovarian tissue were classified in estrus. (b) Cats having one or more corpus luteum on the ovary or ovarian tissue were classified in diestrus.

Results

Estrous stages of queens: According to findings of vaginal cytology, serum E2 and P4 concentrations, and macroscopic examinations, it was determined that 12 cats were in estrus, 14 in diestrus, and 10 in interestrus. It was observed one or more follicles in estrous, and one or more corpus luteum in diestrus as shown in Figure 1 while neither follicle nor corpus luteum was observed in interestrus. Serum E2 and P4 concentrations were in the expected ranges by the classification of reproductive cycle carried out by using vaginal cytology and macroscopic examination. Such that, serum E2 concentrations were 29.18 ± 3.95, 6.32 ± 0.57, and 7.25 ± 1.25 pg/mL while P4 concentrations were 1.70 ± 0.36, 15.14 ± 2.84, and 0.70 ± 0.13 ng/mL in estrus, diestrus, and interestrus, respectively.

Serum thyroid-related hormones concentrations: As shown in Table 1, although the level of the T3 varied depending on the estrous stages, it was not significant according to statistical analysis ($P>0.05$). The highest concentration of thyroxine (16.32 pg/mL) was in cats with interestrus, while the lowest concentration was measured in cats with estrus. Also, slight non-significant change in the blood levels of T4 (P>0.0.5). The T4:T3 ratio changed within a narrow range during the estrous

Table 1. Changes of hormone levels related thyroidal activity of cats at different reproductive stages.

Parameters	Estrous stages	n	Mean	S.E.M	Min.	Max.	P value
T3 pg/mL	Estrus	12	2.44	0.24	1.29	4.43	0.193
	Diestrus	14	2.09	013	1.28	2.99	
	Interestrus	10	2.58	0.23	1.33	3.51	
T4 pg/mL	Estrus	12	14.53	1.00	10.90	20.40	0.552
	Diestrus	14	15.73	1.00	6.90	22.90	
	Interestrus	10	16.32	1.49	9.40	23.50	
T4:T3 ratio	Estrus	12	6.39	0.57	3.37	9.65	0.221
	Diestrus	14	7.77	0.59	3.71	12.11	
	Interestrus	10	6.64	0.67	3.77	9.11	
TSH uIU/mL	Estrus	12	ND	ND	ND	ND	-
	Diestrus	14	ND	ND	ND	ND	
	Interestrus	10	ND	ND	ND	ND	

ND: Not detected

Table 2. Correlations between all variables.

Pearson Correlation	E2 pg/mL	P4 ng/mL	T3 pg/mL	T4 pg/mL	T4:T3
E2 pg/mL	1				
P4 ng/mL	**-0.341***	1			
T3 pg/mL	0.132	-0.168	1		
T4 pg/mL	-0.144	-0.121	0.320	1	
T4:T3	-0.234	-0.033	**-0.629****	**0.481****	1

*: Correlation is significant at the 0.05 level (2-tailed).
**: Correlation is significant at the 0.01 level (2-tailed). Correlation coefficient could not be analyzed on TSH due to the inadequate hormone levels in serum samples of each group

cycle was observed, but not statistically significant (P>0.05). Serum TSH levels of all cats in each phase of estrous were below the detection limit of the assay kit, which could not be compared according to different stages.

Ovarian and thyroid hormones correlations: Correlations coefficient of all measured hormones are shown in Table 2. The thyroid-stimulating hormone could not be included in the analysis of correlation coefficient due to inadequate concentrations in serum samples to detection. A negative correlation was found (r=-0.341, P< 0.05) between the concentration of E2 and P4 in all cats' blood. It was also observed that T4:T3 ratio had a negative relation with T3 (r= -0.629, P< 0.01), and a positive relation with T4 (r= 0.481, P< 0.01).

Discussion and Conclusion

The hormones of the hypothalamic–pituitary–thyroid axis are generally measured to assess the activity of thyroid gland, which can be affected by various physiological and pathological factors. Furthermore, the determination of normal values of these hormones in any physiological status of living beings is critical for the diagnosis of the thyroidal activity. Measurement of free T3 and free T4 show more reliable results than total T3 and total T4 measurements for the evaluation of thyroid dysfunctions (both bound and free) due to the possible changes in plasma level of binding proteins (Feldman et al., 2014). Also, it was noted that the test sensitivity of serum-free T4 is higher than the serum total T4 in terms of hyperthyroidism. Many cats (more than 95%) with hyperthyroidism have increased levels of free T4 while they have levels of total T4 within the reference ranges (Peterson et al., 2001). In this study, serum concentrations of free T3, free T4, T4:T3 ratio, and the relationship between reproductive steroids and thyroid hormones in healthy queens at different reproductive stages have been reported for the first time. Because thyroid-related diseases are more commonly observed in females compared to males, female cats were included the study (Ghani et al., 2017).

Although radioimmunoassay (RIA) is the most suitable method for the measurement of thyroid hormones, different techniques were required because of various methodological limitations of RIA such as radioactive contamination (Vaske et al., 2014). Because serum free T3 and T4 of cats were measured by using a chemiluminescent technique validated for thyroid hormones in sheep (Eshratkhah et al., 2011), horses (Suárez-Esquivel and Castro-Ramírez, 2016), dogs, and cats (Higgs et al., 2014), hormonal analysis in this study was carried out with electrochemiluminescence technique. Results of the present study showed that the variations in free T3 and free T4, and T4:T3 ratio were not statistically significant between different phases of the estrous cycle. Similarly, Dalvi et al. (2013) reported that serum T3 and T4 levels were higher in estrous stages than that of diestrus, however not significant. The authors were also showed a non-significant variation on T4:T3 ratio during the estrous cycle of buffaloes, even if it was higher in diestrus compare to estrus. Following these reports, the fluctuation of T3 and T4 concentrations of goat (Zarei et al., 2009) and mare (Johnson, 1986) during the reproductive cycle were not remarkable. Moreover, Ghani et al. (2017) showed that serum T3 and T4 concentrations in cyclic and acyclic buffaloes were not significantly different. On the other hand, it was reported that ovarian status could affect thyroid activity; significant changes in thyroid hormones in cows were noted during the estrous cycle (Ashkar et al., 2010; Rastogi and Agarwal, 1990; Soliman et al., 1963), and serum T3 and T4 concentrations of bitches were found higher in diestrus than that of proestrus (Reimers et al., 1984).

The connection between the hypothalamus, pituitary, and thyroid glands by the feedback mechanisms controls the thyroidal activity. Therefore, it could be useful to measure blood TSH levels which can influence by ovarian activity. Soliman et al. (1963) reported that thyroid hormone levels in cows were high and thyrotropic hormone level was low during estrous, while thyroid hormone levels were low and thyrotropic hormone level was high during diestrus. However, a higher concentration of circulating T4 results in TSH level reduction. Particularly, the blood level of TSH could be very low in most cats with hyperthyroidism. In a 14-months study, it was reported that hyperthyroidism was detected in only approximately half of the 104 cats with undetectable TSH levels (Wakeling et al., 2011). On the other hand, Peterson et al. (2015) reported that serum TSH levels of 33.6 % of clinically normal and 15.6 % of euthyroid cats in a previous study were below the detectable value, which extend to zero as reference range in normal cats. In the present study, it was assessed the serum TSH concentrations of cats at various reproductive stages. However, the undetectably lower TSH levels found in all cats agree with previous studies mentioned above. Therefore, the measurement of TSH may not be useful for the assessment of thyroidal activity in cats.

According to the results of the present study, there was a negative correlation between E2 and P4 concentrations under the physiological process, in which increased P4 levels suppresses follicular development results in decreased E2 level (Shille and Sojka, 1965). Estrogen deficiency promotes metabolic dysfunction predisposing to obesity, metabolic syndrome, and type 2 diabetes (Mauvais-Jarvis et al., 2013). Estradiol regulates energy homeostasis in the brain in both sexes (Xu and López, 2018). On the other hand, thyroid hormones have various effects on follicular functions and development (Krassas et al., 2010; Wei et al., 2018). It was noted that hypothyroidism decreases the conception rate indirectly due to estrous cycle irregularities or the cessation of estrous cycle (Ghani et al., 2017). Also, Pamela and Richard (2009), reported a close relationship between low thyroid hormones and delayed puberty. It has been also previously reported that hormonal stimulation of ovaries leads to an increase in free T4 levels in cows (Ashkar et al., 2010). In contrast to these reports, it was not observed any correlations between the hormones of the ovary and thyroid gland. This may be most likely related to the type of hormones measured in this study which were free form rather than total form of T3 and T4 since protein-bounded thyroid hormones are more susceptible to physiological and pathological variations compare to free form (Feldman et al., 2014).

The findings of correlation coefficient analysis were showed that the T4:T3 ratio had a negative relation with T3, but a positive relation with T4. This ratio gives information about the conversation of T4 to T3, and it was approximately 15:1 in normal cats (Peterson, 2016). A decrease in this ratio could be related to iodine deficiency while an increase in it could be related to cellular deiodinase type I and II (Maunder et al., 2018). This ratio was found approximately 7:1 in this study. This value was lower compared to the report of Peterson (2016) since only free from of thyroid hormones were assessed which were little amount of total T3 and T4. Free T4 is the only T4 form that can pass across cell membranes to be used as a prohormone for T3 (Vaske et al., 2014). Therefore, it was suggested to be not surprising the close relationship between thyroid hormones and the T4:T3 ratio.

In conclusion, it was observed that non-significant variations on blood concentrations of free T3 and free T4 in domestic female cats at different reproductive stages. Even though It was also measured sera TSH concentrations, it was not within the range limit of assay kits. In addition, although some of the above-mentioned results have been a close relation between ovarium and thyroid gland, correlations between E2, P4, T3, T4, and TSH concentrations of cats were not statistically significant. The thyroidal activity of cats is still taking the attention of veterinary practitioners and researchers. Therefore, further *in vivo* and/or *in vitro* comprehensive studies are required to exhibit the interaction between both glands.

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