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Are cardiac and oxidant-antioxidant status different in female cats in sexual cycle?

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ABSTRACT

This study aimed to investigate the levels of cardiac troponin I (cTnI), malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GSH-Px) during the sexual cycle in domestic female cats. For this purpose, blood samples were collected from twenty-six cats, with an equal number of animals in each group. The cats were divided into two groups luteal and non-luteal periods based on their progesterone levels. Malondialdehyde was used to assess the oxidant status, while GSH and GSH-Px were used to evaluate the antioxidant status. Cardiac troponin I levels were measured to assess cardiac status. The results revealed no significant differences in cardiac troponin I, MDA, or GSH levels between the groups, while GSH-Px levels showed a statistically significant difference. In conclusion, further research is necessary to explore the molecular mechanisms involved in the sexual cycle of domestic female cats

Dişi kedilerde kardiyak ve oksidan-antioksidan durum seksüel siklus dönemlerinde farklı mıdır?

ÖZET

Bu çalışmada seksüel siklus dönemindeki evcil dişi kedilerde kardiyak troponin I (cTnI), malondialdehid (MDA), glutatyon (GSH) ve glutatyon peroksidaz (GSH- Px) düzeylerinin araştırılması amaçlandı. Bu amaç doğrultusunda her grupta eşit sayıda hayvan olacak şekilde yirmi altı adet kediden kan örnekleri alınarak progesteron seviyelerine göre luteal ve non-luteal dönem olmak üzere iki gruba ayrıldı. Elde edilen kan örneklerinde oksidan durum değerlendirilmesi amacıyla MDA, antioksidan durum için GSH, GSH-Px; kardiyak durum için ise kardiyak troponin I seviyeleri ölçüldü. Elde edilen kan örneklerinde kardiyak troponin I, MDA ve GSH seviyelerinin gruplar arası istatistiksel farklılığa neden olmadığı görülürken GSH- Px'in ise iki grup arasında istatistiksel anlamda birbirinden farklı olduğu görüldü. Sonuç olarak evcil dişi kedilerde seksüel siklus dönemindeki moleküler mekanizmaların ortaya konması amacıyla daha ileri araştırmaların yapılması gerekliliği görülmektedir.

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1. Introduction

In recent years, there has been an increase in studies focused on the sexual cycle period in female cats (1,2). Cats are seasonal polyestrous animals that are affected by light and show multiple oestrus during the season and then enter a non-cyclic anoestrous period (3). In addition, cats show provoked ovulation, unlike other seasonal polyestrous animals, and the estrous cycle involves prooestrus, oestrus, interoestrus and dioestrus (if ovulation has occurred) (4). Reproductive hormones such as estrogen (E_2), progesterone (P_4), and androgen fluctuate throughout the sexual cycle (5). In addition, a study revealing the relationship between the fluctuating course of reproductive hormone levels and reproductive receptors in cats revealed changes in estrogen and progesterone receptors during the estrous cycle (6,7). Evidence suggests the existence of multiple regulatory mechanisms connecting nervous, endocrine, and cardiac functions (8). For instance, estrogens play a crucial role in regulating cardiomyocyte functions (9).

A close relationship exists between cardiac function and changes in reproductive hormones during the sexual cycle (8,10). Given the interconnections between the endocrine and cardiovascular systems, hormonal changes inevitably impact cardiovascular function (7,10). It has been reported that cyclic changes in sex hormones controlled by the reproductive cycle in humans or rodents overlap with changes in vascular function (11). A study observed that serum E_2 and P_4 changes affected ventricular premature beats and cardiac repolarisation parameters, and estradiol peak reduced ventricular arrhythmias (12). Also, it has been reported that hormonal changes during the sexual cycle affect cardiac potassium channels (10).

Recognizing the role of oxidative stress in physiological processes, maintaining a precise balance between oxidative and antioxidant states is essential for proper steroidogenesis, angiogenesis, embryo implantation, pregnancy, and childbirth (1,14). It is known that oxidative stress under physiological conditions, in which free radicals increase, affects many reproductive activities such as oocyte maturation, fertilisation, embryo development and pregnancy (13). Antioxidant enzymes, in addition to reproductive hormones, play crucial roles in regulating reproductive functions in cats, and alterations in these enzymes can lead to reproductive dysfunctions such as infertility, anovulation, and abortion (1). Studies on women have reported that oxidative stress is formed under physiological conditions in different menstrual cycle periods (14,15). It has been reported that hypoestrogenism, seen especially after menopause, is associated with high oxidative stress. However, regular fluctuations in estrogen levels occurring during the menstrual cycle do not trigger oxidative stress (16). In addition, another study conducted in women reported that oxidative stress did not change during the menstrual cycle (17). On the other hand, in a study, it was observed that biomarkers of oxidative stress, such as hydrogen peroxide and thiobarbituric acid, were high in the luteal phase of the sexual cycle in women with regular sexual cycles (18). In addition, another study reported that oxidative stress is formed in approximately two-thirds of the menstrual cycle in women (15).

The overpopulation of stray cats, the adoption of cats as pets, and the growing trend of cat breeding in European countries have increased awareness among owners regarding their cats' reproductive physiology (19,20). In addition, the similarities between cat oocytes and human oocytes have attracted the attention of many researchers in the field of reproduction (21). Therefore, studies regarding the feline reproduction biology have grown drastically recently (22). Particularly, the interplay between oxidative stress, steroid hormones, and cardiac function is receiving increasing attention. Estrogens have antioxidant properties, and their deficiency leads to an increase in reactive oxygen species. This phenomenon is observed following ovariohysterectomy in cats (23,24). *Gözer et al.* (23) stated that ovariohysterectomy caused significant changes in the oxidant-antioxidant balance after the ovariohysterectomy in short term while in long term. *Torabi et al.* (24) described that the effect of ovariohysterectomy on oxidant-antioxidant balance is insignificant in long term in cats. Also, the effect of estrogen and progesterone hormones on cardiac function and structures was well described in cats (25,26). In addition, antioxidant enzymes are expressed differentially in the uterus during the estrus cycle of the cats, which indicates the regulatory roles of the antioxidant system on the uterine proliferation (1).

Despite the relationship between the oxidative stress, ovariohysterectomy, and cardiac function, blood cardiac parameters and oxidant-antioxidant enzymes during different periods of the sexual cycle in female cats have not been sufficiently elucidated. This study aimed to investigate the changes in cardiac function and oxidative-antioxidative

status at different stages of the sexual cycle in cats by measuring cardiac troponin I, malondialdehyde (MDA), glutathione (GSH), and glutathione peroxidase (GSH-Px) levels.

2. Material and Methods

Animals

After ethical approvals were obtained, the samples to be used in the study were obtained from animals brought to Hatay Mustafa Kemal University Veterinary Health Practice and Research Hospital Directorate. This study used 6-20-month-old female cats showing oestrus at least once. In the study, after evaluating the animals' general health, cats that showed no gynecological pathological symptoms, had not been administered medroxyprogesterone acetate, had not been in contact with or mated with a male cat, and exhibited regular estrus were included. Also, cats that had previously experienced pyometra and did not show estrus were excluded from the study.

Experimental design and groups of study

After the cats were restrained, blood samples were taken from the v. cephalica. Blood sera were obtained after centrifugation at 5000 rpm for 10 minutes. Progesterone levels were meticulously measured in serum samples, and study groups were formed. In this study, cats were categorized according to their sexual cycle phases into two groups, luteal and non-luteal, with an equal number of animals in each group (n = 26). A P₄ level greater than 1.5 ng/ml was considered a luteal period and included in the luteal group, while those with a P₄ level less than 1.5 ng/ml were determined as non-luteal (27). Progesterone levels were measured using electrochemiluminescence immunoassay test kits on the Atellica IM analyzer (Siemens Healthineers) which has been validated for cats (23,28). The results obtained were expressed as ng/ml.

Cardiac troponin I (cTnI) analysis

Cardiac troponin I (cTnI) levels in the blood samples obtained were measured with an Atellica IM analyzer (Siemens Healthineers) using electrochemiluminescence immunoassay kits. The results obtained were expressed as ng/ml.

MDA, GSH, and GSH-Px analyses

Malondialdehyde, glutathione, and glutathione peroxidase levels in sera obtained from blood samples were measured by spectrometric method. MDA levels, an index of lipid peroxidation, and one of the thiobarbituric acid species were measured according to the method described by *Placer et al.* (29). GSH was measured by the yellow-colored GSH molecules formed by 5,5'-dithiobis (2-nitrobenzoic acid; DTNB) according to *Sedlak and Lindsay's* method (30). GSH-Px was also analyzed according to the method described by *Lawrence et al.* (31). The Shimadzu UV1700 spectrometer was used for these analyses. MDA and GSH were expressed as nmol/ml and GSH-Px as IU/L.

Data analyses

Normality and variance homogeneity tests were performed for all variables obtained. Normality analysis of the variables was performed using the Shapiro-Wilk test, and the Levene test performed homogeneity of variance analysis. For the variables found to be generally distributed as a result of the Shapiro-Wilk test and the variances found to be equal as a result of the Levene test, the differences between the groups were analyzed using the independent sample T-test. Appropriate nonparametric tests (Mann-Whitney U test) were used for variables that did not meet normality or were not homogenous. All statistical analyses were performed at a 5% significance level (p < 0.05). Statistical analyses were performed using IBM SPSS Statistics 23, and all results were expressed as mean ± standard error of the mean (Mean ± SEM).

3. Results

Cardiac troponin I (cTnI) levels

There was no statistically difference in terms of Cardiac troponin I levels between the non-luteal (0.019 ± 0.003 ng/ml) and luteal (0.013 ± 0.002 ng/ml) groups. Cardiac troponin I values are shown in **Figure 1**.

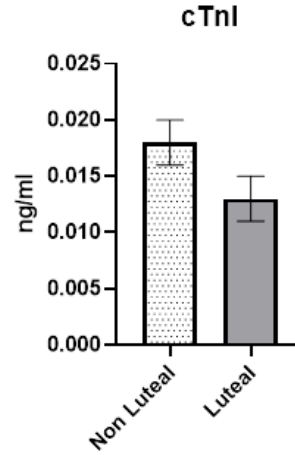


Figure 1: Cardiac troponin I level of non-luteal and luteal groups, (Mean ± Std. Error of Mean)

Şekil 1: Non- luteal ve luteal gruplara ait kardiyak troponin I seviyeleri, (Aritmetik Ort. ± Std. Hata)

MDA, GSH and GSH-Px levels

The MDA levels measured 33.679 ± 0.829 nmol/ml in the non-luteal group and 31.472 ± 0.790 nmol/ml in the luteal group, with no significant statistical difference noted between them. MDA levels are illustrated in Figure 2a.

GSH levels were measured at 3.936 ± 0.047 nmol/ml in the non-luteal group and 4.154 ± 0.096 nmol/ml in the luteal group, with no statistically significant difference between them. GSH levels are depicted in Figure 2b.

Upon comparison of both groups, GSH-Px levels were found to be 158.005 ± 5.413 IU/L in the non-luteal group and 129.401 ± 10.640 IU/L in the luteal group, with a statistically significant difference between the two periods ($p = 0.025$). GSH-Px levels are shown in Figure 2c.

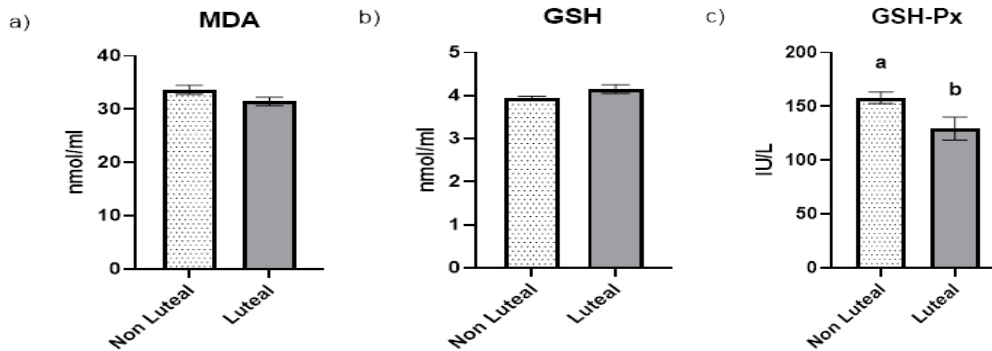


Figure 2: a. Malondialdehyde (MDA), b. Glutathione (GSH), c. Glutathione Peroxidase (GSH-Px) levels in the groups, (Mean ± Std. Error of Mean). The columns' letters indicate statistical differences.

Şekil 2: Gruplara ait a. MDA b. GSH ve c. GSH-Px seviyeleri, (Aritmetik Ort. ± Std. Hata). Sütunlar üzerinde harfler istatistiksel farklılığı ifade etmektedir.

4. Discussion and Conclusion

Sex hormones influence numerous physiological processes, including various cardiovascular functions, with effects that may vary depending on fluctuations in hormone levels during pregnancy or estrus (32). Numerous studies have shown that changes in reproductive hormones cause changes in the cardiovascular system (10,12,33,34). For example, in a study, it was reported that sex hormones have effects on potassium channels in the vascular system (10). In addition, it has been reported that increased estrogen levels in the physiological menstrual cycle in women have inotropic and chronotropic effects (12). It is stated that changing electrocardiography (ECG) findings during a normal menstrual cycle may be related to sex hormones such as estrogen and progesterone, and it may cause opposite effects on the heart (33,35,36). In a study on the QT interval of sex hormones, it was reported that estradiol prolonged the corrected QT interval (QTc), but progesterone showed the opposite effect (34). A study reported that the frequency of ventricular premature beats decreased as the estradiol peak increased during ovulation (12). In addition, it has been demonstrated that estrogen therapy prevents ventricular arrhythmias in postmenopausal women with ventricular premature beats (37). However, it has also been reported that abnormal cardiac pathologies such as arrhythmias and premature beats are not observed in Holter monitoring performed at two different periods of the menstrual cycle in women (8). A study in women found that cardiac output was higher in the mid-luteal phase compared to the mid-follicular phase (38). Additionally, long-term estrogen deficiency has been linked to reduced cardiomyocyte contractility in animal studies (9). Hormonal fluctuations throughout the menstrual cycle are dynamic in women (32). While these menstrual hormonal changes are commonly associated with conditions such as migraines and irritable bowel syndrome, one study observed recurrent elevations in cardiac troponin levels during menstrual periods (39).

The molecular structure of cardiac troponin I is somewhat similar in mammalian species, and cardiac troponins are measured in addition to echocardiography and electrocardiography, providing information about the state of cardiomyocytes in cardiac and non-cardiac patients (40,41). In addition, cardiac biomarkers have been used for many years as part of the clinical evaluation of heart disease, especially in dogs and cats (42,43). In this study, although there was a difference between the mean cardiac troponin levels between the two periods of the sexual cycle in cats, statistical significance was not observed.

It is stated that there is a relationship between estrogen and progesterone, which are reproductive hormones, and oxidative stress, and that this relationship starts near the estrogen peak and is slightly decreased in the progesterone hormone phase; however, this situation is normal under physiological conditions (44). Oxidative stress changes occurring in the phases of the menstrual cycle are related to reproductive hormones such as E₂, P₄, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (14). Oxidative stress peaks during E₂ and LH peaks, while oxidant-antioxidant status is in balance during the period when P₄ is dominant (14). In addition, while E₂ levels and GSH-Px were positively correlated, no correlation was observed with hormones such as FSH, LH, and P₄ (44). A study reported that oxidative stress reaches a peak level, primarily through antioxidant enzymes, during oocyte maturation and possible implantation in the late follicular and early luteal phase of the menstrual cycle (14). In addition, according to oxidative stress analysis performed in plasma samples taken daily during menstruation (27 days) in 20 healthy women, it was stated that oxidative stress was formed in approximately 2/3 of the cycle; however, this situation was physiological, and it was also reported that various phases of the cycle should be examined (15). In a study on the effects of menstrual cycle on redox balance and lipid peroxidation of endometrial tissue in 40 women aged 21-41 years, it was reported that no difference was observed in MDA levels in subjects divided into four menstrual periods as early proliferative, late proliferative, early secretory and late secretory according to endometrial samples, while statistical differences were observed in GSH, GSH-Px depending on the cycle (14). Another study observed that erythrocyte GSH-Px levels increased in the late follicular and luteal phases. In contrast, SOD and catalase levels did not change in the sexual cycle (44). However, in a study conducted in nine healthy women aged 18-44 years with regular sexual cycles, it was reported that there was no change in the oxidative stress status of blood samples taken on different days of the menstrual cycle (17).

A study on female cats was divided into three sexual cycle periods: proestrus/oestrus, dioestrus, and anoestrus. It was reported that the expression levels of superoxide dismutase one and catalase in uterine tissue did not change

during the cyclic periods; however, glutathione peroxidase was expressed lower during dioestrus than other periods (1). Given that the sexual cycle is a dynamic process with fluctuations in reproductive hormones, changes in oxidant and antioxidant status are expected at different stages of the cycle (15). However, a review of some studies reveals that certain oxidative stress biomarkers do not undergo significant changes across the phases of the sexual cycle (14,17,44). Similarly, in this study, MDA and GSH, which are oxidative stress parameters, were not statistically different in different periods of the sexual cycle; however, GSH-Px was statistically significantly higher in the first period of the cycle. This suggests that fluctuations in reproductive hormones may activate distinct oxidant-antioxidant molecular mechanisms; however, further research is needed to clarify these effects.

The present study was limited in several ways. First, the sample size was lower in this study. A larger sample size is needed to elucidate the effect of the cats' sexual period on the cardiac and oxidative stress parameters. Second, although cats are provoked-ovulatory animals which require matings, spontaneous ovulation can be seen in cats due to tactile, olfactory, auditory or visual stimuli (45). In addition, *Chatdarong et al.* (46) reported that adrenocorticotrophic hormone (ACTH) stimulation and routine blood sampling procedures can increase progesterone concentrations in cats. Therefore, a larger sample size is required to rule out the effect of handling-related stress and ovulation on the study design.

As a result, it was observed that cardiac troponin I, MDA and GSH levels were at similar levels in sexual cycle periods (luteal and non-luteal) in cats, but GSH-Px was higher in the non-luteal period compared to the luteal period. Based on these results, it is thought that further studies on this period in cats may provide important contributions to the cardiac and reproductive performances of cats.

Conflict of Interest

The author declared that there is no conflict of interest

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Authors' Contributions

Motivation/Concept: İshak GÖKÇEK

Design: İshak GÖKÇEK, Ahmet GÖZER

Control/Supervision: İshak GÖKÇEK, Ahmet GÖZER

Data Collection and /or Processing: İshak GÖKÇEK, Ahmet GÖZER

Analysis and /or Interpretation: İshak GÖKÇEK, Ahmet GÖZER

Literature Review: İshak GÖKÇEK, Ahmet GÖZER

Writing the Article: İshak GÖKÇEK

Critical Review: İshak GÖKÇEK, Ahmet GÖZER

Ethical Statement

All procedures involving study animals in the experiment were approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision No: 2024/04-05).

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