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A. Katica,¹ N. Mlaćo,¹ M. Katica¹*Original paper***DETERMINING THE LEVEL OF ESTRADIOL CONCENTRATION
IN SERUM OF THE DOMESTIC CATS (FELIS CATUS) OVER THE
PERIOD OF ONE YEAR****Abstract**

Reproductive ability of the domestic cats, based on the experience, varies, which depends on the range of both internal and external factors. Like their wild counterparts, the domestic cats reproduce several times a year; hence, they are polyestric animals. Actuality of endocrinological testing, considering the extreme importance of the ovaries in the neuroendocrinology system and the importance of impact of different factors, climatic factors in particular from the aspect of the onset and detection of estrus, prompted us to determine the frequency of estrus during the period of one year, based on the level of estradiol concentration in blood serum.

Key words: domestic cat, estradiol, estrus.

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*Оригинални рад***ОДРЕЂИВАЊЕ НИВОА КОНЦЕНТРАЦИЈЕ ЕСТРАДИОЛА
У СЕРУМУ ДОМАЋИХ МАЧАКА (FELIS CATUS) ТОКОМ
ПЕРИОДА ОД ЈЕДНЕ ГОДИНЕ****Кратак садржај**

Репродуктивна способност домаће мачке, на основу искуства, варира, што зависи од опсега интерних и екстерних фактора. Као и њихови дивљи сродници,

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домаће мачке репродукују неколико пута годишње. Стога, оне су полиестричне животиње. Актуелност ендокринолошких тестирања, с обзиром на изузетну важност јајника у неуроендокринолошком систему и значај утицаја различитих фактора, климатских фактора посебно, са аспекта настанка и откривања еструса, навело нас је да утврдимо учесталост еструса током периода од једне године, на основу нивоа концентрације естрадиола у крвном серуму.

Кључне ријечи: домаћа мачка, естрадиол, еструс.

INTRODUCTION/ УВОД

Sexual maturity in domestic cats is conditioned by the existence of functional ovaries, and they are the ovaries in which Graafian (tertiary follicles) mature and then burst and eject a fertilizable egg into the oviduct. All these events take place in the ovaries of exclusively sexually mature animals. The ovaries are inactive up to the puberty. On their surface, there are numerous primary follicles and during the reproductive period, only small number of them will develop and mature. A double role of the ovaries begins with sexual maturing: female sex cells-oocytes are maturing in them and secretion of female sex hormones, estrogens and progesterone, begins.

The main ovarian estrogen hormone is estradiol. The examination of the hormonal status of estradiol in peripheral blood of cats is particularly important for precise detection of the onset of estrus.

Estrogens isolated from urine – estrone and estriol – have slightly weaker action than estradiol, and, in fact, they are its metabolic products. Besides being

responsible for the onset of estrus, estradiol also boosts the development of female sex characteristics. Inactivation of this hormone occurs in liver, and to some smaller extent, in kidneys.

Considering the fact that cats are polyestric animals, i.e. their onset can happen throughout the entire year, which, of course, depends on the living conditions, climatic factors and isolation in particular, the goal of our research is to determine the intensity of sexual cyclicity based on the results received.

MATERIAL AND METHODS/ МАТЕРИЈАЛ И МЕТОДЕ

The material required for the research, i.e. whole blood, was taken from 26 domestic cats that were kept indoors, age from one to three years old. These were sexually mature animals, females, which were not in direct contact with males. The animals were in good health condition; their body temperature, pulse and breathing were in physiological ranges. Our study concerning the level of estradiol concentrations in blood serum in cats involved two time intervals during

the year, from January to July and from October until middle December, hence during the estrus season and outside the mating season. The blood for the analysis was taken in the beginning, in the middle and in the end of the research periods, from different animals that had been feed in different ways.

Blood samples of 5 ml coagulated after 30 minutes, and then, they were centrifuged at 3000 turns for one minute. The extracted serum was deposited in the freezer at -20°C from the beginning of the laboratory testing. 24 hours prior to lab testing the serums were brought to 4°C , and then to room temperature.

After that, the serums were homogenized, but the levels of the estradiol and progesterone concentrations were determined using DELFIA method.

DELFLIA method is a Fluoroimmunoassay with non-radioactive marker, which uses fluorescence for reading the results of the immunological reactions.

Marking of antigens or antibodies is done using fluorescent marker from the group of lanthanides – europium. Since in its complex it has a very weak fluorescence, following the immunological reaction, its dissociation occurs, and it creates a chelate in a special solution, which has a million times stronger fluorescence.

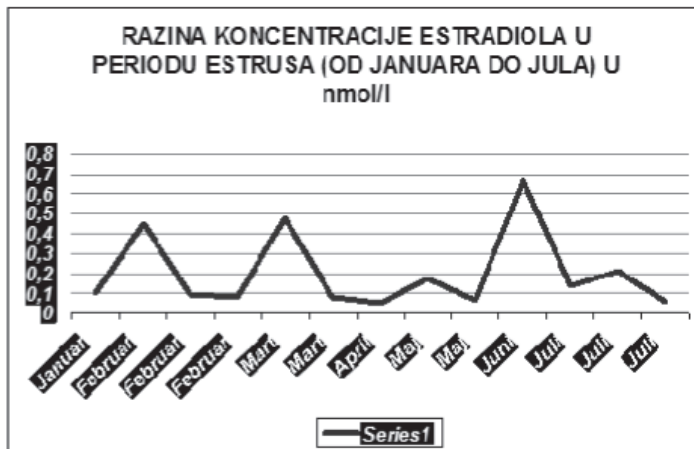
RESULTS AND DISCUSSION/ РЕЗУЛТАТИ И ДИСКУСИЈА

The results were shown using graphs in order to visually observe variations of the estradiol concentration during the ti-

me intervals significant for reproduction of the domestic cats. We used the original results, expressed in $\text{nmo}1/1$.

First, we will address the levels of estradiol in serum of cats during estrus. The curve for estradiol level during the year has ups and downs. The values of the estradiol significantly declined from January, and then in February, the concentration was $0,077 \text{ nmo}1/1$ on average (Graph 1). The lowest concentration of estradiol in serum was observed in February. It was $0,045 \text{ nmo}1/1$. The average monthly temperature for that period was $5,8^{\circ}\text{C}$, and insolation lasted 108,2 hours. However, the levels of estradiol concentration in the same month, in February, also showed not so insignificant variety; from the minimum value of $0,045$ to $0,93 \text{ nmo}1/1$. The curve for estradiol level abruptly rose; in March, it reached the value of $0,28 \text{ nmo}1/1$. In addition, during the same month (March), the values of the estradiol concentrations varied similar to those in February, from $0,083$ to $0,48 \text{ nmo}1/1$. In April, the curve significantly drops, that is, the estradiol concentration dropped down to $0,05 \text{ nmo}1/1$. In May, the curve goes up to the average of $0,124 \text{ nmo}1/1$ and continues to rise to maximum values in June, $0,67 \text{ nmo}1/1$.

The average monthly temperature for this period was $19,4^{\circ}\text{C}$, and insolation lasted 188,0 hours. The estradiol concentration abruptly declines as of June, and in the following month it was at $0,138 \text{ nmol/l}$, that is, it varied from $0,061$ to $0,213 \text{ nmol/l}$ (Graph 1).

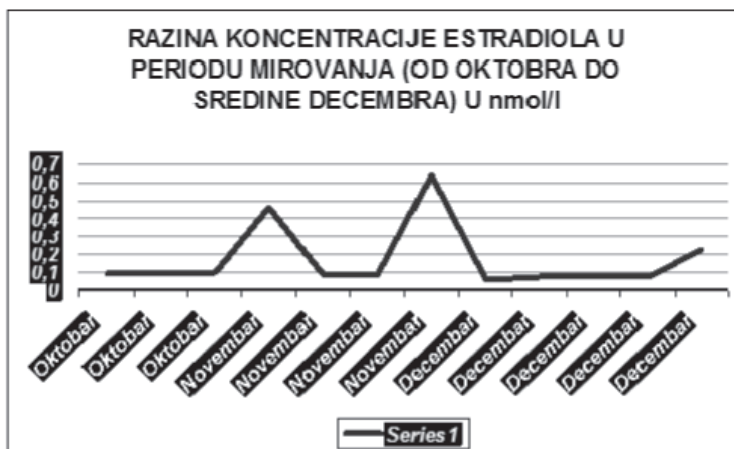


Graph 1. Levels of Estradiol concentrations during period of Oestrus (from January to July)

During the time known as the time of “inaction” or abstinence, which is from October to mid December, there is significant variation in the levels of estradiol concentration in the serum of tested animals. In October, the average concentration of estradiol was 0,093 nmol/l, i.e., it ranged from 0,088 to 0,1 nmol/l (Graph 2). In the following month of November, the level of estradiol in serum abruptly rises to the maximum-

recorded value of 0,319 nmol/l. This time is also characterized by variations in determined concentrations, ranging from the minimum values of 0,079 nmol/l to the maximum level of estradiol – 0,466 nmol/l.

The minimum value of the estradiol concentration 0,057 nmol/l (Graph 2), was recorded in December when the average monthly air temperature was 2,4C°, and insolation 40,9 hours.



Graph 2. Levels of Estradiol concentrations during silent period (from October to December)

In November, which was rather warm, with average monthly air temperature of $9,8^{\circ}\text{C}$, and insolation of 107,1 hours, we recorded the highest concentration of estradiol 0,466 nmo1/1, which is close to the level of estradiol concentration recorded during estrus time, that is in March, and which was the maximum of 0,48 nmo1/1.

It is known that estrogens cause estrus in all species, although, in some animals, small amounts of progesterone can also play a certain role in inducing estrus (Jo Ann Eurell and Brian L. Frappier, 2006). Prior to estrus, for a period of few days, follicles, Graafian in particular, significantly rise, mature. In that case, there is an increased follicular liquid rich in hormones (A. Katica et al., 2010). Estrogens, mostly 17β estradiol and estrone, produce theca interna cells that surround tertiary follicles in growth, helped with gonadotrophin hormones of pituitary (A. Mutevelić et al., 2003; Junqueira and Carneiro, J., 2005). The estradiol and progesterone concentrations will vary depending on the sexual cycle phase (Taylor D., 1988; Tsutsui et al., 1993). Hence, the authors claim that the estradiol concentrations at the moment of mating is around 60 pg/ml, while in the next five days, the estradiol concentration level drop down to 8 to 12 pg/ml.

Other authors (Taha, M. B. and Noakes, D. E., 1982) examined the impact of age and seasonality on the function of testes and level of testosterone concentrations. They determined that the levels of testosterone concentrations gradually rose during estrus regardless of

the age of animals, especially in spring and autumn. We compared the authors' observations with our research results, regardless of the fact that they had treated male animals. Considering that in our research we did not strictly pay attention to the age of the cats (in line with authors cited), however, these were sexually mature animals, the estradiol concentration during estrus gradually rose from Feb to July.

The drop of estradiol concentration was observed from the end of May to the beginning of June. Furthermore, with the drop in air temperature and insolation, the estradiol concentration gradually drops from Oct to Nov, only to see the concentration rise again at the beginning of Dec. The research also determined that the level of estradiol concentration in blood serum of the animals varied both during estrus and outside the mating season. It is normal that the level of estradiol concentration shows different values, which depends on the functional condition of the ovaries, i.e. whether it is the follicular, luteal phase or the middle of sexual cycle (Verhage et al., 1976; Shille et al., 1979; Burkit et al., 1993), which corresponded with the findings of our research.

CONCLUSIONS/ ЗАКЉУЧЦИ

Based on examining the level of estradiol concentration in blood serum of domestic cats during one year, we concluded the following:

- The level of estradiol concentration both in estrus and outside the mating season shows some variations.

- Maximum values of the levels of estradiol concentration during estrus were observed in June at 0,67 nmol/l, while the minimum value was in February at 0,045 nmol/l.
- The period outside estrus, from October to mid December, is characterized by maximum values of estradiol in November at 0,466 nmol/l, while the minimum value was in December at 0,057 nmol/l.
- Considering the different ways of feeding of domestic cats, there are some deviations in the levels of estradiol concentrations during the research periods. Somewhat higher concentrations of estradiol in December in relation to February might be linked with the different ways of feeding and care for the researched animals.
- With reducing insulation and air temperature, the level of the estradiol concentration gradually declines; however, it begins to rise in late autumn, as of December.

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