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PROGESTERONE PROFILE IN THE SEXUAL CYCLE OF FEMALE AMERICAN MINK (*NEOVISON VISON*)

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Abstract. The aim of this study was to analyze the blood serum levels of progesterone in American mink females in the course of the annual sexual cycle. In order to determine the concentration profile of progesterone in year-old females of two color varieties, Pearl (P) and Standard Black, short NAP (BV), blood was collected from a representative group of 16 randomly selected females. The frequency of blood sampling was variable depending on the phase of the cycle of the studied females. Blood was collected once a month from late May to September (early anestrus), every 14 days from October to December (late anestrus) and in January and February (gonadal preparation and re-activation period). Analysis of the blood serum concentrations of progesterone in female mink indicates a clear downward trend from May to October followed by lowest values remaining until late February.

Key words: American mink, progesterone, sexual cycle

INTRODUCTION

The American mink (*Neovison vison*) is a monoestrous animal with the pituitary gonadotropin activity largely depending on changes in light regime, which – according to Papke et al. [1980] and Amstislavsky and Ternovskaya [2000] – define the beginning of the breeding season, as well as embryo implantation and development time. The dependence was noted as early as in 1962 by Holcomb et al. [1962].

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The sexual cycle of female mink can be divided into three subsequent phases. It starts with the resting period, further divided into early period, from late May to late September when primordial follicles are of maximum size 0.4–0.6 mm [Sundquvist et al. 1989, Klotchkov and Eryuchenkov 2003, Persson 2007], and late resting period, from October to December, characterized by a slight swelling of the vulva [Pilbeam et al. 1979]. During this period, the outer cortex of the ovary contains primary follicles surrounded by a single layer of follicular cells [Sundquvist et al. 1989]. In addition, as indicated by Klotchkov and Eryuchenkov [2003], the beginning of the breeding season is characterized by a higher number of follicles that start maturation. Another phase is the proestrus, lasting from January to February. According to Vitale et al. [2001], this phase is characterized by an increased production of follicle stimulating hormone (FSH) – secreted by the anterior lobe of the pituitary, responsible for stimulating maturation of ovarian follicles. According to Travis et al. [1978] and Pilbeam et al. [1979], also strong swelling of the vulva can also be observed in this phase. The last phase is the estrus [Holcomb et al. 1962], in which there is the maximum swelling of the vulva and constant readiness to mate [Pilbeam et al. 1979]. Furthermore, there is a continuous growth in size of follicles beyond 0.7 mm; as is apparent from the experiments by Douglas et al. [1994] only follicles of such size become dominant, mature and ovulate.

Reproductive season in the northern hemisphere begins when day becomes about two hours longer than the eight-hour winter day [Boissin-Agasse et al. 1996, Klotchkov et al. 1998, Felska-Błaszczyk and Sulik 2008, Felska-Błaszczyk et al. 2010]. There are, however, some differences in duration of the breeding season reported by some authors; e.g. Adams [1981], Stoufflet et al. [1989], Lagerkvist et al. [1992],Toumi et al. [1992] or Gulevich et al. [1995] state that the breeding season may begin as early as late February and take about four weeks. According to Fink et al. [1998] and Matthiesen et al. [2010], it lasts about three weeks in March, while Holcom et al. [1962], Sundqvist et al. [1989], Tauson et al. [2004] and Felska-Błaszczyk and Sulik [2008] state that breeding season lasts entire March. According to Persson [2007], a breeding season can lag up to early April. Undoubtedly, difficulties in unambiguous estimation of the length of the breeding season arise from the fact that – according to Felska-Blaszczyk et al. [2010] – the heat cycle starts differently depending on the color morph of the female. An interesting hypothesis proposed by Garciá [2010] suggests that the wild American mink are likely to have two breeding seasons. The author's conclusions are based on his observations of mink mating in August.

Due to the numerous discrepancies regarding the mink sexual cycle, the aim of this study was to measure and analyze the levels of progesterone in the blood serum of female American mink in the course of the annual sexual cycle.

MATERIAL AND METHODS

The trial took place on one of the largest mink farms located in West Pomeranian Voivodeship in Poland. The animals were housed in multi-purpose two-row mink sheds with Danish-type cages, combined in sets of 8. The animals were fed semi-liquid feed based on chicken and fish, in a conventional way, according to widely adapted standards. The feed was placed on top of the cages using a feed dispenser.

In order to measure serum progesterone concentrations, we collected blood from 16 randomly assigned year-old females representing two color morphs: Perl (P) and Standard Black Velvet (BV), also refered to as short NAP.

The frequency of blood sampling depended on the phase of the sexual cycle of the females. Blood was collected once a month between late May and September (early anestrus), and fortnightly from October to December (late anestrus) and in January and February (gonadal preparation and re-activation period).

Blood was obtained by clipping the toenail of a front or hind leg, the technique commonly used in for Aleutian disease antibody testing. A toenail of a temporily immobilized female was cut slightly above the vein line using disinfected clippers, and 200 µl of blood was collected directly to a heparinized sample tube. No anaesthetics were used during the operation to avoid hormonal release disturbances, which could affect the results. The blood samplings were performed on designated dates (Table 1) between 9.00 a.m. and 11.00 a.m., and the samples were centrifuged and stored frozen until analysis.

Table 1. Dates of blood collections for analysis of progesterone concentration profile

Tabela 1. Terminy pobrania próbek krwi do analizy profilu stężenia progesteronu

Color morph Odmiana barwna	Blood sampling dates Terminy pobrania krwi													
P	19 V	22 VI	24 VII	25 VIII	22 IX	6 X	21 X	6 XI	20 XI	4 XII	21 XII	4 I	20 I	2 II	18 II
BV	19 V	22 VI	24 VII	25 VIII	22 IX	6 X	21 X	6 XI	20 XI	4 XII	21 XII	4 I	20 I	2 II	18 II

The concentration of the hormone was determined by immunofluorescence assay using the Delfia® kit (Perkin-Elmer Wallac Oy, Turku, Finland). This test is based on the competition for antibody binding sites between europe^{+3}-labeled hormone and unlabeled hormone contained in the sample. The amount of antibodies of the labeled hormone is constant, while the content of unlabeled hormone is a function of antibody – labeled hormone complex formation. On this basis, a standard curve was prepared and used to read hormone concentration in the sample.

Statistical analysis of the results was carried out using the STATISTICA 10.0 PL package.

RESULTS AND DISCUSSION

As a result of this analysis, no statistically significant differences between the analyzed mink color varieties in serum progesterone concentrations were found. The analysis shows a distinct downward trend starting from 19 May, with the highest average value of 1.9 ng · ml^{-1}. The trend continued until 21 October, to remain at a level of 0.6 ng · ml^{-1} afterwards. Next, a quite clearly marked period of lowest and relatively stable averages could be observed, which ranged within 0.4–0.5 ng · ml^{-1} (Figs. 1, 2).

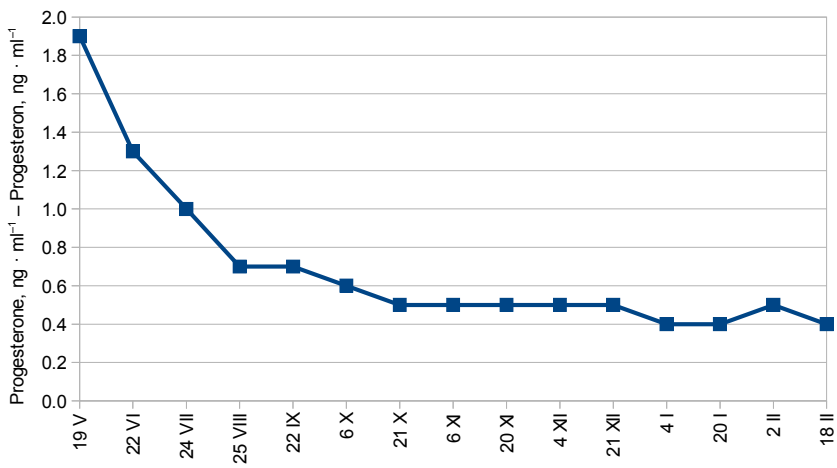

Fig. 1. Blood serum progesterone on different sampling dates

Rys. 1. Stężenie progesteronu w surowicy krwi w zależności od terminu jej pobrania

Evaluation of differences in progesterone levels between the two analyzed color morphs indicates the presence of higher concentrations among Pearl females in 8 of 15 blood samplings. The differences have not been confirmed statistically, though (Fig. 2).

Reports by numerous authors who studied progesterone profiles in mink focus on pregnant females. According to Adams [1981], the level of progesterone in the first days after mating remains at 8.0 ng · ml^{-1}, which is followed by an increase from 51.0 ng · ml^{-1} at 10 days after mating to 99.2 ng · ml^{-1} seven days later, as reported by Bäcklin et al. [1997], who studied Standard Black females. Felska-Błaszczyk et al. [2011] found a more than threefold increase in blood level

of progesterone in female mink between 25 March and 8 April, with the averages 13.49 ng · ml^{-1} and 49.78 ng · ml^{-1}, respectively. The peak values of blood progesterone in female mink range between 72 and 160 ng · ml^{-1}, depending on the source [Møller 1973, Allais and Martinet 1978]. Once they are reached, progesterone levels gradually decrease until birth [Papke et al. 1980, Sundquvist et al. 1989]. According to Verhage et al. [1976], a similar progesterone profile, increasing until the moment of implantation or soon afterwards and gradually decreasing until the end of pregnancy, occurs in the cat.

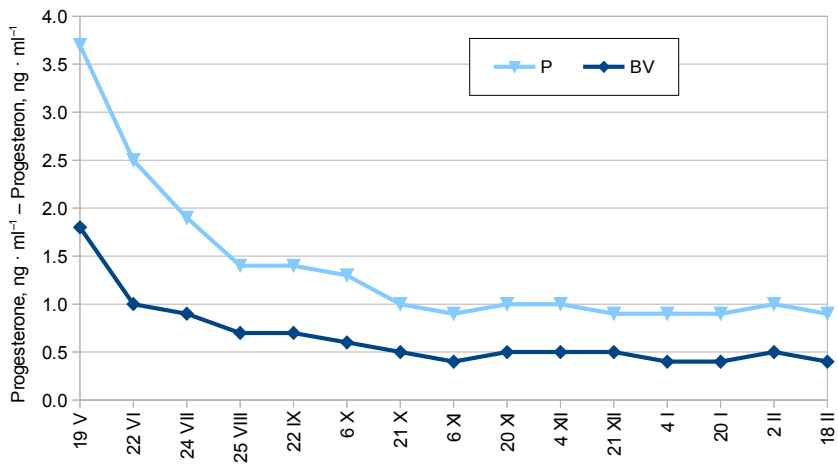

Fig. 2. Blood serum progesterone concentrations by color morph and sampling date

Rys. 2. Stężenie progesteronu w surowicy krwi zależności od odmiany barwnej norek i terminu jej pobrania

The concentrations of blood serum progesterone in the sexual cycle of female American mink found in our study, which remain at a very low level until end of February, do not correspond with the results by Pilbeam et al. [1979]. The authors claim that gonad activity remains in regression in spring, which is at the absolute minimum during summer and remains so until late fall.

CONCLUSION

Analysis of the blood serum concentrations of progesterone in female mink indicates a clear downward trend from May to October followed by minimum levels remaining until late February.

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OKREŚLENIE PROFILU PROGESTERONU W PRZEBIEGU CYKLU PŁCIOWEGO SAMIC NORKI AMERYKAŃSKIEJ (*NEOVISON VISON*)

Streszczenie. Celem pracy była analiza stężenia progesteronu w surowicy krwi samic norek amerykańskich w trakcie przebiegu rocznego cyklu płciowego. W celu określenia profilu stężenia progesteronu u jednorocznych, samic dwóch odmian barwnych: perła (P) i standard czarny short NAP (BV), pobrana została krew od reprezentatywnej grupy 16. losowo wybranych samic. Częstotliwość pobierania krwi była zmienna w zależności od fazy cyklu płciowego, w jakim znajdowały się poddane badaniu samice. Raz w miesiącu krew pobierana była od końca maja do września (okres wczesnego anestrus), natomiast co 14 dni w pozostałych miesiącach roku – od października do grudnia (okres późnego anestrus) oraz w styczniu i lutym w okresie, w którym następuje przygotowanie i wznowienie czynności gonad. Analiza uzyskanych wyników dotyczących stężenia progesteronu w surowicy krwi samic norek wskazuje na wyraźną tendencję spadkową wspomnianego parametru w okresie od maja do października oraz utrzymania najniższych stwierdzonych wartości do drugiej połowy lutego.

Słowa kluczowe: norka amerykańska, progesteron, cykl płciowy

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