INTRODUCTION

Given the scale of production and the quality of harvested pelts, mink are the most important fur animals in Poland’s fur farming. Currently, Poland ranks fourth in the world’s mink pelt production. In the mink, a monoestrous species, the estrus occurs in March, when days become longer. When the light phase of the day reaches 10 hours, neurohormonal stimulation of the gonads and the ova takes place [Hammond 1951, Pilbeam et al. 1979, Wehrenberg et al. 1992]. The length of the estrus shows great interindividual variability and lasts from several days to three weeks (10–14 days on average). Ovulation in the mink is induced and occurs most often between 36 and 72 hours after mating [Venge 1973, Wehrenberg et al. 1992]. Maturation of oocytes during the heat takes place in two to four cycles, every 7–8 days.
pause, that is an interruption in embryonic development at the blastocyst stage and a delayed implantation, is a commonplace phenomenon in female mink. Delayed implantation can lead to another cycle of egg cell maturation. Various mating patterns are in use on mink farms; however, each of the systems recommends a repeated mating on the following day, as well as another one in 7–8 days. The length of gestation in mink, due to a varying duration of diapause, depends among other factors on the mating date (females mated earlier have a longer gestation, whilst those mated later have a shorter one).

Implantation of the embryo is regulated by prolactin, a hormone released by the glandular part of the pituitary. The release is triggered by estrogen, which in turn activates progesterone release from a corpus luteum, enabling implantation of the embryo in the uterus wall [Murphy et al. 1983, Tauson et al. 2000].

Progesterone and testosterone are important hormones, whose concentrations change during the pregnancy. Testosterone in males is produced by interstitial cells of Leydig located in the testes, in the presence of LH, and also in small amounts by the adrenal cortex; in females the hormone is released from the ovaries and placenta [Matt and MacDonald 1984]. An adult male body produces about ten times more testosterone than the mature female body, but the female body is more sensitive to changes in the levels of this hormone. In healthy ovarian follicles, androgens perform two main functions. Firstly, acting via a classical receptor-mediated pathway, they regulate follicular development in a paracrine/autocrine manner; secondly, through binding to cytochrome P450arom, they serve as substrates for estrogen production [Szolty et al. 2005]. However, the range of testosterone concentrations in either females or males is so wide that the lower margin of the range in males and the top one in females overlap. In females, the peak levels of testosterone in the blood occur in the final days of follicular phase of the estrous cycle, which leads to the onset of ovulation.

Progesterone is involved in the estrous cycle in females, pregnancy, and embryogenesis. This hormone, produced by corpora lutea, is one of the most important hormones secreted by the ovaries. Secretion of progesterone increases after ovulation, preparing the endometrium for the reception of a fertilized egg, and halting further maturation of follicles. Progesterone exerts its primary action through the intracellular progesterone receptors [Luconi et al. 1998, Yang and Yi 2000]. Progesterone is sometimes referred to as the “pregnancy hormone” (when it is secreted by the placenta), because it plays a key role in maintaining pregnancy (by inhibiting uterine contractions) and in the normal course of fetal development. According to Felska-Błaszczyk et al. [2011], plasma concentrations of progesterone in female mink mated during heat can be used as a pregnancy indicator.

The concentration of progesterone in pregnancy varies and depend on the species. In cats, for most of gestation, progesterone concentrations remain at a similar level as during the heat, and then drop sharply 1–2 days prior to delivery [Verhage et al. 1976]. In small ruminants, its level during pregnancy tends to increase, which depends on the number of fetuses. In dogs, the concentration initially increases, remains at a constant level over the next 15–20 days, and then slowly decreases until birth [Özyurtlu et al. 2006]. The concentration of progesterone in pregnant cows, compared with the period of heat, is increased and remains at constant level throughout the pregnancy. Matt and MacDonald [1984] found

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in rats that the placenta produces progesterone and testosterone ceaselessly during pregnancy. Studies in bitches have shown that progesterone and testosterone levels during pregnancy are positively correlated, and the concentrations of progesterone and testosterone change simultaneously [Gudermuth et al. 1998].

The aim of this study was to analyze plasma concentrations of progesterone and testosterone in pregnant mink and the relationship between these hormones in different stages of pregnancy, in relation to the color variety of the females.

MATERIALS AND METHODS

The study was carried out on a mink farm in western Poland. All animals were equally fed wet fish-based feed, and water was constantly available from automatic drinkers. Blood was collected from females in order to determine the level of progesterone (P4) and testosterone (T). Due to the fact that the method of blood collection is invasive, the number of animals used for sampling was limited to fourteen pregnant mink females: seven Wild-type Standard Brown females and seven Black Velvet females, also referred to as Short NAP. The mink of either color variety were at age of one year.

Blood sampling and collection of biological material

Blood samplings took place following the permission of the Local Ethical Committee for Experiments on Animals. Blood was collected seven times on the following dates:

1) 5 March, directly prior to the first mating,
2) 10 March,
3) 17 March,
4) 19 March,
5) 27 March,
6) 3 April,
7) 8 April, approx. 3 weeks before expected parturition.

Blood was drawn from under the toenails, according to the procedures commonly used in the diagnosis of Aleutian disease. The animal was manually immobilized for a moment (no anesthetic was used, since it could disturb the hormone levels) and a toenail of a hind limb was clipped with previously disinfected clippers above the vein line. Each time, 2–3 ml of blood was collected and placed in heparin-containing test-tubes. The samplings took place in the morning, between 9.00 and 11.30. The blood was centrifuged and the plasma was stored at −18°C until analysis.

Determination of progesterone and testosterone concentrations

Hormone concentrations were determined by immunofluorescence using the Delfi® kits (PerkinElmer, Turku, Finland), which is based on the fluorescence of elements. The Delfi test is based on the competition for binding sites on the antibody molecule that occurs between the Europium¹³-labeled hormone and a not-labeled hormone, contained in the sample. The amount of the labeled hormone is constant, whilst the not-labeled hormone
content is a function of antibody-labeled hormone complex formation. On this basis, a standard curve was drawn for reading the hormone levels in the sample.

**Statistical analysis**

Differences in groups depending on the variety of color and date of collection was found by using the Statistica® 7.0 PL., using multivariate analysis of variance (ANOVA) in the orthogonal design. Mathematical analysis included the mean (m) and standard deviation (SD). This analysis was based on the following linear model:

\[ Y_{ijk} = m + o_i + w_j + (ow)_{ij} + e_{ijk} \]

where:
- \( Y_{ijk} \) – value of a given trait,
- \( m \) – total mean of the trait,
- \( o_i \) – effect of blood collection date,
- \( w_j \) – effect of color variety,
- \( (ow)_{ij} \) – interaction: date of collection x color variety,
- \( e_{ijk} \) – random error.

Using the Statistica® 7.0 PL package, Spearman rank correlations were calculated between the concentration of progesterone (P4) and testosterone (T).

**RESULTS AND DISCUSSION**

Due to a large number of significant differences found, the data on the effect of blood sampling date and the effect of the color variety were separated into two tables for the sake of picture clarity. The results concerning the level of progesterone and testosterone in the blood plasma of pregnant mink depending on the date of blood collection are shown in Table 1.

Statistical analysis revealed a number of significant differences (at \( P \leq 0.01 \) and \( P < 0.05 \)) between the subsequent dates of blood collection. The data shows that progesterone concentration ranged from 0.733 to 59.857 ng per ml in Black females and from 0.737 to 68.100 ng · ml⁻¹ in Standard Brown females. In both color varieties the lowest amount of progesterone was reported on 5 March, just before mating, whereas the highest value was observed on 3 April. Testosterone was at the level of 0.004 to 0.292 ng · ml⁻¹ in Black females, while in Standards Brown females at the level of 0.007 to 0.290 ng · ml⁻¹. The lowest level of this hormone were recorded on 5 March, the moment prior to mating.

Figures 1 and 2 visualize the changes in the level of steroid hormones, P4 and T, in the blood plasma of pregnant mink depending on the date of blood collection in two color varieties, Black, so-called short NAP, and Standard Brown, Wild type.

Table 2 lists the values for progesterone and testosterone concentrations, depending on the color variety. Like in the analysis of the relationship between the date of blood collection and the concentration of hormones in the blood plasma of pregnant mink, signifi-
Plasma concentrations of progesterone and testosterone in pregnant mink (*Neovison vison*)...

cant relationships progesterone and testosterone concentrations were found in two varieties of colored females. In the case of progesterone, significant (at P ≤ 0.01) differences in concentration depending on the variety of color was observed on 27 March and 3 April, while in the case of testosterone on 27 March (Table 2).

Table 1. Concentration of progesterone and testosterone in various stages of pregnancy, depending on the time of blood collection

<table>
<thead>
<tr>
<th>Color variety</th>
<th>Date of sampling Data pobrania</th>
<th>Concentration, ng·ml⁻¹ – Stężenie, ng·ml⁻¹</th>
<th>progesterone</th>
<th>testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black, short NAP</td>
<td>1 5.03</td>
<td>0.733&lt;sup&gt;ABC&lt;/sup&gt; 0.250 0.004&lt;sup&gt;ABD&lt;/sup&gt; 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Czarna, tzw. short NAP</td>
<td>2 10.03</td>
<td>2.111&lt;sup&gt;DEF&lt;/sup&gt; 0.680 0.039&lt;sup&gt;G&lt;/sup&gt; 0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 17.03</td>
<td>2.991&lt;sup&gt;GH&lt;/sup&gt; 0.692 0.156&lt;sup&gt;AB&lt;/sup&gt; 0.182</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 19.03</td>
<td>4.130&lt;sup&gt;RL&lt;/sup&gt; 0.821 0.292&lt;sup&gt;BC&lt;/sup&gt; 0.182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Brown, Wild</td>
<td>5 27.03</td>
<td>33.929&lt;sup&gt;AGDLM&lt;/sup&gt; 11.102 0.195&lt;sup&gt;G&lt;/sup&gt; 0.047</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard brązowy, wild</td>
<td>6 3.04</td>
<td>59.85&lt;sup&gt;BEKL&lt;/sup&gt; 6.260 0.168&lt;sup&gt;DF&lt;/sup&gt; 0.101</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 8.04</td>
<td>57.59&lt;sup&gt;CFLM&lt;/sup&gt; 8.468 0.13&lt;sup&gt;EF&lt;/sup&gt; 0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Brown, Wild</td>
<td>1 5.03</td>
<td>0.737&lt;sup&gt;ABC&lt;/sup&gt; 0.090 0.007&lt;sup&gt;ABCD&lt;/sup&gt; 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>2 10.03</td>
<td>2.226&lt;sup&gt;DEF&lt;/sup&gt; 0.555 0.038&lt;sup&gt;G&lt;/sup&gt; 0.071</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 17.03</td>
<td>4.234&lt;sup&gt;GH&lt;/sup&gt; 1.413 0.109&lt;sup&gt;AB&lt;/sup&gt; 0.062</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4 19.03</td>
<td>4.849&lt;sup&gt;RL&lt;/sup&gt; 1.838 0.212&lt;sup&gt;BC&lt;/sup&gt; 0.187</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 27.03</td>
<td>44.771&lt;sup&gt;AGDLM&lt;/sup&gt; 13.881 0.235&lt;sup&gt;G&lt;/sup&gt; 0.125</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6 3.04</td>
<td>68.100&lt;sup&gt;BEKL&lt;/sup&gt; 5.425 0.290&lt;sup&gt;DF&lt;/sup&gt; 0.118</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>7 8.04</td>
<td>59.557&lt;sup&gt;CFLM&lt;/sup&gt; 12.545 0.175&lt;sup&gt;EF&lt;/sup&gt; 0.094</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A, B, C, ... – differences statistically significant at P ≤ 0.01 – różnica statystycznie istotna na poziomie P ≤ 0.01.

a, b, c, ... – differences statistically significant at P ≤ 0.05 – różnica statystycznie istotna na poziomie P ≤ 0.05.

Based on the results of analysis with Statistica® 7.0 PL, using Spearman’s rank order, we found a positive correlation between serum progesterone and testosterone in pregnant mink, which amounted to 0.68.

Statistically significant differences indicate that the concentration of both the analyzed hormones depended on the date of blood collection. Prior to mating, progesterone concentration was very low. Rouvinen-Watt et al. [2010] reported plasma concentration of progesterone in blood collected in January at the level of 1.195 nmol·l⁻¹ (equivalent to 0.325 pg·ml⁻¹) in female Black mink at the age of 9 months. The highest significant differences in progesterone concentrations were observed between samples obtained before mating (collection 1), and samples obtained from the same animals after 19 March (dates
In the case of testosterone, the highest differences in its concentration in blood were observed between the samples from before mating (collection 1), and the samples collected on 19th March (sampling 4), probably just before the end of diapause, and samples taken on 27th March and 3 April (collection 5 and 6), probably after the implantation of the embryo in the uterine wall. In both cases, one can therefore conclude that hormone levels change with the development of the fetus.

Fig. 1. Concentration of progesterone and testosterone in pregnant Black (short NAP) mink by the date of blood collection, ng·ml\(^{-1}\).

After fertilization, the embryo develops for about 6–8 days until the stage of blastocyst containing 200–500 cells [Moreau et al. 1996, Desmarais et al. 2004]. At this stage, the embryo passes into the uterus, where its further development ceases (diapause) [Murphy et al. 1983, Wehrenberg et al. 1992, Ferguson et al. 1996]. During diapause, mitotic divisions of the embryo are inhibited and its implantation is delayed in time [Song et al. 1998, Lopes et al. 2003]. The size of cells in blastocyst begins to change after implantation, when the reactivation of embryonic development takes place. Diapause period in mink, according to various authors, ranges between 5–6 days to 55 days. The average length of diapause is 18–25 days [Murphy and James 1974].
Plasma concentrations of progesterone and testosterone in pregnant mink (*Neovison vison*)...

Fig. 2. Concentration of progesterone and testosterone in pregnant Standard Brown (Wild) mink by the date of blood collection, ng · ml⁻¹

Rys. 2. Stężenie progesteronu i testosteronu u norki standard brązowy w zależności od terminu pobrania krwi, ng · ml⁻¹

Table 2. Blood plasma progesterone and testosterone levels by color variety, ng · ml⁻¹

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Date of collection</th>
<th>Color variety – Odmiana barwna</th>
<th>Progesterone, ng ml⁻¹</th>
<th>Testosterone, ng ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.03 0.733</td>
<td>5.03 0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.03 2.111</td>
<td>10.03 0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.03 2.991</td>
<td>17.03 0.156</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.03 33.929</td>
<td>27.03 0.195</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.04 59.857</td>
<td>3.04 0.168</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>5.03</td>
<td>Black, short NAP czarna tzw. short NAP</td>
<td>0.737</td>
<td>0.007</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>10.03</td>
<td>Standard Brown, Wild standard brązowy tzw. wild</td>
<td>2.226</td>
<td>0.038</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>17.03</td>
<td></td>
<td>4.23</td>
<td>0.11</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>27.03</td>
<td></td>
<td>4.849</td>
<td>0.212</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>3.04</td>
<td></td>
<td>68.100^a</td>
<td>0.290</td>
</tr>
<tr>
<td>Progesterone, ng ml⁻¹</td>
<td>8.04</td>
<td></td>
<td>59.557</td>
<td>0.175</td>
</tr>
</tbody>
</table>

A, B – difference statistically significant at P ≤ 0.01 – różnica statystycznie istotna na poziomie P ≤ 0.01.
During the diapause, suspended are any rapid increases in the concentrations of the hormones responsible for the development of the embryo and the maintenance of pregnancy [Stoufflet et al. 1989, Rozhnov et al. 2007]. Shortly after the diapause and implantation of the embryo in the uterus, an increased production of hormones responsible for further development of the fetus and for the maintenance and proper course of pregnancy can be observed. Also, we can observe a decrease in progesterone and testosterone concentrations in the samples from the last blood collection. This may be due to the fact that P4 concentration, after an initial period of growth, begins to decline slowly, preparing the mother and the fetus for birth. Such changes have been confirmed by studies conducted by Özyurtlu et al. [2006] on dogs, with the difference that the authors found also high levels of progesterone persisting for 15–20 days in bitches, which was not observed in mink.

The positive correlation between progesterone and testosterone serum levels have been confirmed by studies conducted on pregnant females of other species, including dogs [Gudermuth et al. 1998], porcupines [Wyk et al. 1994], and voles [Nubbemeyer 1999]. Both the studies on hormone levels during estrous cycle and pregnancy in the vole (maximum concentration of T and P4 during proestrus) [Nubbemeyer 1999], as well as those of progesterone and testosterone concentrations in the serum of female porcupines, have revealed that there is a correlation between the concentration of T and P4 [Wyk et al. 1994]. Also, Lea et al. [1976] found that progesterone and testosterone secretions in pregnant guinea pigs are correlated, since both hormones showed a sharp rise and then fall on the same days of gestation.

CONCLUSION

The overall conclusion is that the concentration of progesterone and testosterone in the blood of pregnant mink significantly depends on the date of blood collection, and hence the period of gestation, as well as on fur color variety. Since these tests were based on determinations in the peripheral blood, it is difficult to determine whether these hormones are largely of placental origin, or have another source. The concentration of progesterone and testosterone in the plasma of pregnant mink also statistically depended on the variety of color. Concentrations of progesterone and testosterone in the blood plasma of pregnant mink were positively correlated with each other.

REFERENCES


Plasma concentrations of progesterone and testosterone in pregnant mink (*Neovison vison*)... 19


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STĘŻENIE PROGESTERONU I TESTOSTERONU W OSOCZU KRWI CIĘŻARNYCH NOREK (NEOVISON VISON) W ZALEŻNOŚCI OD ODMIANY BARWNEJ I TERMINU POBRANIA KRWI

Streszczenie. Celem pracy było określenie stężenia progesteronu i testosteronu w osoczu krwi pobranej w różnych terminach od ciężarnych norek różnych odmian barwnych. Krew pobrano od czternaściu ciężarnych samic norek: siedmiu samic odmiany standardowej brązowej typu wild i siedmiu samic odmiany czarnej, tzw. short NAP. Krew pobierano siedmiokrotnie – pierwszy raz przed rozpoczęciem kryć, a ostatni raz na około trzy tygodnie przed porodem. Stężenia hormonów oznaczono metodą immunofluorescencyjną przy użyciu zestawów firmy Delfia® Perkin-Elmer Wallac Oy, Turku (Finland) z wykorzystaniem zjawiska fluorescencji pierwiastków. Stężenie progesteronu i testosteronu we krwi pobranej od samic w różnych terminach różniło się statystycznie istotnie. Wykazano statystycznie istotne różnice w stężeniu progesteronu i testosteronu w osoczu krwi ciężarnych norek w zależności od odmiany barwnej. Stężenie obydwu badanych hormonów w osoczu ciężarnych norek było ze sobą dodatnio skorelowane.

Słowa kluczowe: norka amerykańska, progesteron, rozród, testosteron

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