Pregnancy Detection in Putatively Unmated Mink (*Mustela vison*) by Serum Progesterone Level

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Abstract. The aim of this study was to determine whether blood plasma progesterone is a reliable indicator of pregnancy in mink at an early stage of gestation. We also attempted to establish the threshold value of progesterone as a pregnancy indicator. The analysis was carried out at a production farm on 42 standard female mink aged 1 year, which were grouped both according to the observed success of mating (“mated” and “unmated”) and the level of blood serum progesterone measured afterwards (“pregnant” and “nonpregnant”). It was next verified whether a particular female had been assigned to the proper group in the first place. An analysis of accuracy of mating success assessment within the group of unmated females revealed that more than one-third of decisions were wrong, since some females that had been considered unmated ultimately whelped. This suggests that mating supervision by farm workers lacks reliability. A progesterone test for verification of such management decisions should limit the risk of error. We suggest that progesterone tests could be carried out using the threshold values 1.9 ng/ml and 20 ng/ml in blood sampled on 25 March and 8 April, respectively, although some level of uncertainty should be taken into account.

Key words: Mink, *Mustela vison*, Pregnancy detection, Progesterone

The sector of mink farming in Poland has been growing significantly over recent years. Due to its high profitability, this area of animal production now represents a very important branch of the agriculture industry. Mink farms often represent very large facilities, which provide housing for herds reaching several thousand females in size. This scale of management allows farmers to carry out extensive breeding programs, aimed also at improvement of reproduction performance.

The American mink (*Mustela vison*) is a monoestrous species, which means it gives offspring only once a year. This fact, along with the presence of the diapause and, in consequence, high embryonic mortality rates, are particular constraints that hamper improvement in the productivity of the animals. In Poland, mink are mated between the 3rd and 21st of March. Females are mated several times during the heat cycle, since follicular development is cyclic; follicles intensively develop in 2–4 waves every 6–8 days. A successful mating of a mink does not mean that new follicles stop to grow, which in consequence allows them to mate throughout the heat cycle [1–3]. Ovulation in mink is provoked by the act of copulation, although there have been suggestions that spontaneous ovulations might occur as well [4]. After ovulation, the ovary develops a corpus luteum, which is responsible for progesterone production. At the first fertilization, the corpus luteum produces very small amounts of progesterone, which does not prevent growth of new follicles. As a consequence, subsequent matings can be carried out. Progesterone is the hormone responsible for both implantation of the embryo in the uterus and sustainment of pregnancy [5]. The level of serum progesterone in the pregnant mink begins to increase approx. 40 days prior to whelping [6], whereas its maximum concentration has been observed at the beginning of April, which is when implantation takes place [7, 8]. After that, serum progesterone declines gradually until parturition.

There is a large amount of data in the literature on blood progesterone levels in pregnant [9–11] and nonpregnant [4] female mink. To date, however, serum progesterone profiles have not been studied in females that, although mated, did not conceive due to an unknown reason. Hence, the pattern of progesterone release in such females, which are usually classified by farmers as “infertile,” is unclear. If we establish the level of progesterone in successfully mated females and those considered “unmated,” we might be able to elaborate a method of excluding pregnancies in mink.

Breeding procedures applied on large fur farms specify that both males and (putatively) unmated females be culled immediately on completion of the mating season, since managing the animals until the next fur maturity season would be economically unjustified. In the case of males, the decision is simple; for females, however, early culling could be a wrong and costly decision. Namely, some of the females – although recorded as “unmated” – ultimately prove pregnant and may give birth to their offspring within a regular time frame. Such cases might result from improper supervision of the mating process. In order to avoid such mistakes, as well as to prevent the loss resulting from the unnecessary housing of infertile females for another 7 months, it seems justified that a diagnostic method should be developed to detect pregnancies in mink.

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The aim of this study was to determine whether the blood plasma progesterone level can be used to confirm or rule out pregnancy in mink as soon as possible after completion of the mating season and to possibly establish the threshold value of progesterone as a pregnancy indicator.

**Material and Methods**

The study was carried out on a mink farm located in north west Poland. The analysis involved 42 standard (wild type) female mink aged 1 year, managed in a production cycle and fed the same ration of fish-based wet feed with drinking water ad libitum. All the mink were housed in the same shed, which provided an equal environment for each female. According to the workers operating the farm, one group of the studied females had been successfully mated (“mated” group, n=21, females no. 22–42), whereas the other group had not been stimulated despite many trials (“unmated” group, n=21, females no. 1–21). The “mated” females were paired with a male twice, at a one-week interval, whereas the group of “unmated” females were bred many times without a positive outcome noted by the farm workers. The animals of the latter group were subsequently intended for culling; however, we refrained from this operation until the time at which all the females that had actually been fertilized gave birth to young. This allowed us to verify whether or not a particular female had been appropriately assigned to its group in the first place.

As a result, when the whelping season was completed, the females were grouped again, and this time the females were grouped as “pregnant” (those that produced a litter, n=29) and “nonpregnant” (those that failed to give birth to young, n=13).

In order to measure the level of plasma progesterone, blood samples were collected from the females. The samplings took place twice, on 25 March and 8 April, 2008. Blood was drawn from a toenail of a rear paw, in accordance with the procedure applied in Aleutian disease testing [12]. No anesthetics were used during blood collection, since it could have affected hormonal secretion and thus cause a bias in the results. The blood collection methodology was approved by the Local Ethics Committee for Animal Research Projects. Progesterone concentration was measured by immunofluorometric assays using Delphia kits (Perkin Elmer, Turku, Finland).

Statistical differences between the concentrations of progesterone in mink that whelped and those that did not were tested with the Mann-Whitney U test using the Statistica 8.0#PL software package.

### Results

Blood progesterone concentrations measured on 25 March were significantly (P≤0.001) lower compared with those measured on 8 April, irrespective of the gestational status of the female (Table 1). In the samples collected on the latter day, the progesterone concentration was nearly three-fold higher, as compared with that in the

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**Table 1.** Blood progesterone in female mink on 25 March and 8 April

<table>
<thead>
<tr>
<th>Gestational status</th>
<th>n</th>
<th>Blood progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 March</td>
</tr>
<tr>
<td>Pregnant</td>
<td>29</td>
<td>12.24± 7.09</td>
</tr>
<tr>
<td>Not pregnant</td>
<td>13</td>
<td>16.29± 15.45</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>13.49± 10.38</td>
</tr>
</tbody>
</table>

Means marked with the same letters, A, B or C, differ (P≤0.001).

**Table 2.** Accuracy of mating success evaluations by farm workers

<table>
<thead>
<tr>
<th>Workers’ assessment</th>
<th>Whelping females, n (%)</th>
<th>Nonwhelping females, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mated (n = 21)</td>
<td>21 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Unmated (n = 21)</td>
<td>8 (38.1%)</td>
<td>13 (61.9%)</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Mean blood progesterone levels by date of sampling and gestational status of females.
samples collected two weeks earlier. In the females that ultimately whelped, the difference was even higher, more than four times (Fig. 1). Those that did not whelp, on the other hand, had twice as much progesterone in their blood on 8 April as on 25 March, though the difference was not statistically significant.

Determination of the progesterone threshold level that could be used to confirm or rule out pregnancy proved difficult. In females whose blood was tested on 25 March, a level of 1.9 ng/ml allowed successful prediction of pregnancy in 37 females (88.09%) and definite elimination of pregnancy in 5 females (11.90%) out of 42 females (Table 2). Eight females with progesterone above 1.9 ng/ml, hence considered pregnant, did not whelp (Fig. 2). Elimination of pregnancy by the applied threshold (< 1.9 ng/ml) was accurate in 100% of cases. In all, 80.95% of diagnoses (confirmation or elimination of pregnancy) were correct.

The accuracy of pregnancy detection based on blood progesterone level is presented in Table 3. On the latter date, i.e. on 8 April, 20 ng/ml was the applied progesterone threshold level that was used to decide whether a female was to be qualified as pregnant or nonpregnant. In this case, none of the females that had had less than 20 ng/ml of progesterone gave birth to offspring. On the other hand, the aforementioned 8 females out of those with a progesterone above this level (thus considered pregnant) did not produce offspring.

Comparison of the evaluation of mating success by the farm workers with the actual results of whelping (Table 2) reveals an interesting observation.

All the females qualified by the workers as mated gave birth to offspring. On the other hand, 38.1% of the workers’ predictions were inaccurate, since females considered unmated also produced offspring.

Discussion

According to Rozhnov et al. [8] and Amstislavsky and Ternovskaya [13], the highest progesterone level in pregnant mink, 29 to 34 ng/ml, is observed at the beginning of April, at the moment of embryo implantation. Similar data were reported by Pilbeam et al. [7], who also stated that the peak progesterone level, 25.5 ± 6.6 ng/ml, could be observed on 8 April. Einarssson [10] observed that the progesterone concentration rises above the level of 10 ng/ml 31–36 days before parturition. According to Allais and Martinet [9], the progesterone level reaches its maximum between 25 and 30 March, i.e., about 10 days prior to embryo implantation in the uterus and 40 days before the expected birth.

The about two-week discrepancy as to the reported date of the
maximum plasma progesterone level prompted us to undertake two blood collections, i.e., on 25 March and 8 April. Eventually, the collection on 25 March did not reveal a significant difference in progesterone level between the mated and putatively unmated females (Table 1). Moreover, on 25 March, the females considered unmated exhibited higher progesterone concentrations, and it seems that the hormone is not a reliable indicator of pregnancy in the early stage of gestation.

Lagerkvist et al. [14] compared the levels of blood progesterone in mated and unmated mink on dates similar to those presented in this study. Unmated females had a much lower concentration of progesterone both on 25 March and at the beginning of April, as compared with mated females. According to this author, the progesterone level in unmated females on the previous date ranged from 0 to 9 nmol/l and ranged from 1 to 6 nmol/l on 7 April; in mated females, however, the levels recorded on the latter date were much higher, exceeding 55 nmol/l. The cited data are similar to what we observed in our investigation; however, in many cases, we found a high progesterone concentration in unmated females.

Of the eight females that were unmated (probably unstimulated) proved to be nonpregnant (Table 3, “nonwhelping”) and had a plasma progesterone level above the threshold values on both sampling days, five (Fig. 2, females no. 4, 8, 9, 18 and 19) demonstrated a pattern of progesterone changes similar to that observed in most of the females that eventually gave birth to young. The difference in the progesterone levels of the remaining three females (Fig. 2, females no. 2, 16 and 20) between 25 March and 8 April was much lower than the difference between the respective means (Fig. 1, left graph). On the other hand, females no. 2, 16, 19 and 20 demonstrated considerably high progesterone on 25 March, exceeding 30 ng/ml, well beyond that expected for a pregnant female that actually produced offspring (Fig. 2). In terms of progesterone-based pregnancy tests, this high level on 25 March should be considered as a high threshold value that allows ruling out of pregnancy at an early stage; however, additional studies should be undertaken in order to confirm or refute this. Females no. 7, 10, 15 and 17, whose levels of progesterone on both dates were close to 0 ng/ml, were actually unmated or were in fact sterile.

If we look closer at the accuracy of the progesterone-based pregnancy testing, we can assume that not all the false positives resulted from the fact that the females were nonpregnant. One could hypothesize that the lack of litters from some of the females with an elevated progesterone level as measured soon after mating, which otherwise indicates pregnancy, might have resulted either from an actual pregnancy terminated by spontaneous mortality of embryos or from spontaneous ovulation.

The lower level of progesterone observed in pregnant females on 25 March (Table 1), along with the aforementioned high level of progesterone in those females that actually were not pregnant, may reflect the hypothesis of Holocomb [15] that excessive progesterone release in the beginning of gestation might lead to embryo reabsorption. This was also described by Franklin [11], who observed that mortality of embryos increases with the duration of diapause in the mink. Embryo deaths might explain the pattern of progesterone changes in females no. 2, 8, 16, 19 and 20 (Fig. 2), which never whelped. However, since all the females classified as “mated” eventually got pregnant and gave birth to young, it is unlikely that embryo mortality was an underlying cause of sterility of some of our females. Also, we cannot confirm that embryo mortality actually took place. A more likely cause of the high progesterone measured on 25 March in putatively unmated and actually non whelping females is that they had ovulated despite the lack of stimulation.

Møller [4] suggests that unmated (i.e., unstimulated) females may ovulate spontaneously and develop corpora lutea that secrete progesterone. It seems that spontaneous ovulation may lead to a pregnancy-like progesterone pattern without the female actually being pregnant. Moreover, it has been suggested that luteinization might occur without ovulation due to death of the ovum [4].

Managing females that fail to produce a litter until the subsequent breeding season considerably reduces the profitability of the farming cycle. A progesterone-based test that would allow saving pregnant, false unmated females from culling would be a valuable tool in farm management and would improve its efficiency.

The progesterone test used for early detection of pregnancy has been applied to other species, such as goats in the study by Baran et al. [16]. As a threshold value, the authors applied the level of 1 ng/ml. The accuracy of pregnancy detection ranged from 54.1 to 100%.

The analysis of the accuracy of mating success assessment done by the workers of the farm within the group of unmated females revealed that more than one-third of decisions were wrong (Table 2), since some females that had been considered unmated finally produced offspring. This implies that observations carried out by farm workers lack reliability. A progesterone test for verification of such management decisions should limit the risk of error; however, some additional research should be done before issuing any recommendations. Namely, a progesterone pregnancy test could be carried out in mink with a threshold value 1.9 ng/ml on 25 March or a threshold value of 20 ng/ml on 8 April (solid and dotted horizon-
terial lines, respectively, in Fig. 2), although neither threshold value guarantees 100% certainty. Also, a high threshold value (30 ng/ml on 25 March) above which the female is likely to be barren could also be considered; however, only 4 females (nearly 10% of the total) showed this level of progesterone.

References