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MATING SYSTEM AND LEVEL OF REPRODUCTIVE PERFORMANCE IN MINK (*NEOVISON VISON*)*

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Abstract

The aim of the study was to examine the degree to which the mating system of female mink determines the level of reproductive performance in this species, to find out if this parameter significantly affects litter size, and if so, which of the systems used on farms is optimal. A total of 1626 mink of the foundation stock were observed. Analysis was made of seven different systems differing in the number of matings and in the mating interval of the female. The effect of mating systems on the level of reproductive performance was tested by multivariate analysis of variance using least square means. The mating scheme, which in this case defined both the number of matings and the mating interval, was a factor that had a highly significant effect on litter size. Regardless of the number of matings of the female, the most favourable level of reproductive performance was characteristic of the mink in which the interval between the first and last mating was the shortest. When choosing the mating system, care must be taken to ensure that it does not prolong the diapause period, which at the same time should reduce the losses associated with embryo death and resorption.

Key words: mink, mating system, reproduction, delayed implantation, embryonic diapause

Mink reproduction has been discussed extensively in the world literature (Berglund, 1986; Elofson et al., 1989; Isakova, 2007; Lefevre et al., 2008, 2009; Møller, 1997; Rozempolska-Rucińska et al., 2004; Sulik et al., 2007; Sundqvist et al., 1989; Ślaska et al., 2009; Yamaguchi et al., 2004). This stems from the fact that mink have a peculiar reproductive physiology, mainly due to the possibility of diapause, but also from the economic dependence of the farm on reproductive performance. To ensure satisfactory reproductive performance in mink, both genetic factors and a number of environmental factors that determine prolificacy must be considered. One of the more important factors is the female mating system in its broad sense. Available research provides no conclusive answers, but clearly indicates that this factor determines reproductive performance in this animal species (Rozempolska-Rucińska

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et al., 2004). Given the current knowledge of delayed embryo implantation and embryonic diapause in mink, it seems necessary to verify the mating system currently used on the farms. Therefore, the aim of this study was to determine the level of reproductive performance in mink according to the mating system of females and, if possible, to offer optimum solutions.

Material and methods

Data for the study were obtained from breeding records of a farm in the Zachodniopomorskie province. Observations were made on 1626 mink of the foundation stock, including about 58% and 42% of one- and two-year-old females, respectively. Animals represented 7 colour types (Mahogen, Palomino, Pearl Cross, Sapphire Cross, Silver, Scanblack and Scanbrown). Farmed females were mated several times: twice (36.90% of mink), three times (28.84%), four times (23.68%), and five times (10.58%). The following mating schemes were used: 1+1; 1+1+1; 1+1+1+1 (females mated on 2, 3 and 4 consecutive days); 1+1+9 (triple mating on two consecutive days, the last mating with an 8-day mating interval); 1+9+10 (triple mating with a 9- and 10-day mating interval); 1+1+9+10 (quadruple mating on two consecutive days and two consecutive matings with an 8- and 9-day mating interval). In addition to these mating systems, the farmed mink were also mated five times without a predetermined system. For this reason, females mated five times were included in one group in the analysis, regardless of the mating scheme. The percentage of female mink in different mating schemes was as follows: 35.81 (1+1); 2.96 (1+1+1); 0.82 (1+1+9); 25.42 (1+9+10); 0.82 (1+1+1+1); 23.35 (1+1+9+10); and 10.82% (quintuple mating).

Mink were also grouped into 3 classes according to the number of days between the first and last mating: 1 to 4 days; 5 to 8 days; 9 days and above.

The analyses included the number of kits born and the number of kits weaned per litter. The level of reproductive performance was analysed by multivariate analysis of variance using the least squares method, taking into account the fixed effects of female coat colour and age, mating system, number of matings, number of days between the first and last mating, and number of matings × mating system interaction.

The calculations were made using SAS statistical package (SAS Institute Inc., 2000). The values of the analysed traits are shown as least square means (lsm) while providing standard errors (se) that determine the reliability of estimation.

The housing and feeding conditions on the farm remained unchanged. The herd was fed in accordance with standards for carnivorous fur animals. Animals were also subjected to necessary preventive and veterinary treatments.

Results

Tables 1 and 2 present the reproductive results of the mink depending on the number of matings and the mating scheme.

The number of matings had a significant effect on the number of kits born and reared per litter.

Significantly the highest reproductive parameters were recorded in the group of females mated twice. In the other groups, prolificacy and percent of kits reared were significantly lower. Poorest reproductive parameters (with significant differences) occurred in the group of females mated three times and five times (Table 1).

Table 1. Reproductive performance of female mink with regard to number of matings*

No. of matings	Born		Reared	
	lsm	se	lsm	se
2	7.42 a	0.09	7.11 a	0.09
3	6.65 c	0.10	6.38 c	0.10
4	6.96 b	0.09	6.67 b	0.10
5	6.86 bc	0.13	6.47 bc	0.13

*Means in columns with different letters differ significantly at $P \leq 0.05$.

There were statistically significant differences in prolificacy and in the number of kits weaned in different mating systems (Table 2).

Regardless of the number of matings, significantly the highest values for both the number of kits born and weaned were found for females mated in the 1+1+1+1, 1+1 and 1+1+1 systems. Considering all the mating schemes under analysis, significantly the lowest values of the analysed reproductive traits were noted for triple mating but only in the 1+1+9 and 1+9+10 systems (Table 2).

For triple mating, significantly the highest values of the analysed reproductive traits were found for the 1+1+1 mating scheme. Females mated three times using the other schemes were characterized by significantly poorer prolificacy and number of kits weaned (Table 2). For quadruple mating, significantly higher reproductive parameters were recorded among females mated on four consecutive days (1+1+1+1) compared to the prolificacy and number of kits weaned from females mated using the 1+1+9+10 system.

Table 2. Reproductive performance of female mink with regard to mating system*

No. of matings	Mating scheme	Born		Reared	
		lsm	se	lsm	se
2	1+1	7.42 a	0.09	7.11 a	0.09
3	1+1+1	7.31 ab	0.25	7.14 a	0.25
	1+1+9	6.13 c	0.46	5.91 c	0.47
	1+9+10	6.55 c	0.10	6.26 c	0.10
4	1+1+1+1	8.00 a	0.47	7.79 a	0.48
	1+1+9+10	6.91 bc	0.09	6.62 b	0.09
5	various	6.86 bc	0.13	6.47 bc	0.13

* Means in columns with different letters differ significantly at $P \leq 0.05$.

Number of days between the first and last mating was the factor that had a significant effect on whelping date (Table 3). Significantly the highest prolificacy and number of kits weaned were established in the group of females with the smallest number of days between the first and last mating (1–4 days). When the mating interval was 9 days and above, female reproductive parameters were significantly lower compared to mink mated on 2, 3 and 4 consecutive days. Significantly the lowest prolificacy and number of kits weaned were established in females with 5 to 8 days between the first and last mating.

Table 3. Reproductive performance of female mink with regard to length of mating period during one mating season*

No. of days between first and last mating	Born		Reared	
	lsm	se	lsm	se
1–4	7.47 a	0.09	7.16 a	0.09
5–8	5.81 c	0.27	5.61 c	0.28
9 and above	6.78 b	0.07	6.47 b	0.07

* Means in columns with different letters differ significantly at $P \leq 0.05$.

Discussion

The number of matings had a significant effect on reproductive parameters of female mink (Table 1), but the results showed that the increased number of matings did not have any effect on prolificacy. In several cases, the results obtained are contradictory to the reports of other authors. Lorek et al. (1994) found triple mating of mink on two oestrus dates to improve conception rate expressed as litter size at birth. Also Møller (1997) reported that repeated mating may have influence on litter size by increasing it by 0.2–0.3 kits. Similarly, Ślaska et al. (2009) found a significantly lower level of reproductive performance in one-year-old female mink representing different colour types when mated twice compared to more than twice. The effect of number of matings on reproductive performance in different colour varieties of female mink was investigated by Sulik et al. (2007), who found that the number of repeated matings for optimum litter size cannot be determined collectively for many colour varieties studied. It should be added, however, that their model included the number of matings rather than the mating scheme, which may have affected the results. Only the findings of Rozempolska-Rucińska et al. (2004) are similar to our results. The authors cited above found that considering reproductive performance of mink, early beginning and early termination of mating is most advantageous, whereas early beginning and late termination of matings may be particularly disadvantageous.

Based on most of the research results quoted above, it might seem that every additional mating of mink, considering the peculiar characteristics of its oestrous cycle, should increase prolificacy. However, no such relationship was found in the population studied. In our study (Table 1), significantly the best reproductive performance was characteristic of twice-mated females. However, knowing the complex reproductive physiology of mink, including the peculiar characteristics of its oestrous cycle, the question arises if it matters whether matings follow on consecutive days or take place at several-day intervals. Both strategies are physiologically justified.

Mating mink on consecutive days may be justified by the interval between mating and ovulation. The mink has induced ovulation, which occurs about 48 h (28–72 h) after copulation (Sundqvist et al., 1989). Mink are mated on consecutive days to increase the likelihood of fertile mating in the event that too much time has elapsed from copulation and fertilization fails due to reduced viability of spermatozoa.

Another interesting physiological phenomenon in the mink is superfecundation, which allows the mink to be fertilized again during one mating period due to the possibility of second ovulation. This means that even if fertilization takes place, the oestrous cycle may continue during embryonic diapause (Yamaguchi et al., 2006). The mink is capable of multiple ovulations within one mating season. During March, ovarian follicles may mature four times at about 8-day intervals. If the female is fertilized during the first mating, she may still ovulate 8–10 days later, and thereafter may give birth to kits from two different ovulations, a phenomenon known as superfetation (Sundqvist et al., 1989). This means that conception (fertilization) takes place during pregnancy (Yamaguchi et al., 2006). Repeated fertilization is associated with delayed implantation (DI) of embryos into the uterus, known as embryonic diapause. During pregnancy, lutein cells of the corpus luteum produce progesterone, but the mink differs from other species in that corpora lutea cannot initially produce high quantities of progesterone. This low level of progesterone allows a second oestrus, and the ovulation of a second set of eggs capable of normal development after fertilization (Sundqvist et al., 1989).

Elofson et al. (1989) found that mating in the 1+9 system (double mating with a 9-day interval between the matings) allows for another ovulation to occur. Thus, mating systems utilizing the phenomenon of repeated ovulations during the breeding season have been applied to the mink (Sundqvist et al., 1989). From the physiological point of view, the theory that prolificacy increases when the mink is mated with an 8-day interval between matings, is fully justified. This is due to the phenomenon found in many species of animals. The phenomenon of diapause is characteristic of several orders of mammals, but it is common to the *Mustelidae* family. They account for over half of all mammals in which delayed fertilization has been described (Thom et al., 2004). Embryonic diapause (delayed fertilization) is the reversible arrest of mammalian embryo development prior to implantation, due to suppression of cell proliferation at the blastocyst stage (Lefevre et al., 2008, 2009). In the mink, embryonic diapause occurs during every pregnancy, while reinitiation of development is associated with increased endometrial secretion. Thus, the resumption of embryo development is triggered by secreted uterine factors (Lefevre et al., 2009). Lefevre et al. (2008, 2009) used a gene library to determine genes showing different expression during diapause and reactivation of blastocyst development in the uterus of mink. They selected candidate genes potentially responsible for resumption of embryo development after implantation in the uterus. It is believed that mutations within these genes may contribute to the lack of gene expression and, as a consequence, to the lack of embryo development after delayed implantation in the uterus. However, there is no information in the literature concerning the expression of candidate genes in the event of short and long diapause. Without elucidating this issue, it can only be assumed that the expression of candidate genes may be different during prolonged diapause, which may be another reason for the absence of embryo development after implantation.

The significant differences observed for reproductive results of females mated using different systems may be attributed to the prolonged diapause period and the delayed implantation of blastocysts. Blastocysts cannot develop until the end of the mating season, thus increasing the risk of embryo death and reducing female prolificacy. According to Sundqvist et al. (1989), ova fertilized after the last ovulation develop more often than ova from previous ovulations.

Berglund (1986) concluded that most losses during the prenatal period occur between fertilization and implantation of embryos in the uterus, and the risk of losses increases with the lengthening of this period. Therefore, the unfavourable effect of the number of days between the first and last mating is likely attributable to high mortality of embryos before their implantation in the uterus (Elofson et al., 1989) and after the implantation (Lefevre et al., 2008, 2009).

Based on the findings of different authors cited above, both theories should hold true in farm practice. This would mean that regardless of whether matings occur day after day or at several-day intervals, they should produce benefits in the form of high prolificacy. But is this really so? Our findings indicate that these physiological phenomena are not reflected in breeding practice. To state more precisely, there must be other unknown factors at work that differentiate female prolificacy on the farms depending on the mating system. The results presented in Table 2 show that reproductive parameters are significantly correlated not only with the number of matings but also with the mating scheme. Analysis of female prolificacy and the number of kits weaned reveals an interesting relationship. Significantly the best reproductive performance was characteristic of females mated on consecutive days. When females were mated in a system accounting for the second ovulation, i.e. with a several-day mating interval, the mink were characterized by significantly lower reproductive performance. This fact may suggest that diapause had a negative effect on reproductive performance. This conclusion is supported by the results presented in Table 3. Significantly the lowest reproductive performance of females in which the interval between the first and last mating was 5–8 days, is indisputable. This is due to the fact that the mink were mated successively in the period with no ovulation (Sundqvist et al., 1989), which means that additional matings during this period should not increase prolificacy. Mention should be made, however, of the presence of corpora lutea in unmated females that had been separated from males several months before the breeding season. This suggests the possibility of spontaneous ovulation in mink (Sundqvist et al., 1989). Our study shows, however, that this factor is not significant enough to increase the level of reproductive performance in mink.

The previous discussion about the negative effect of diapause on mink prolificacy is confirmed by the presence of significant differences between reproductive parameters of females in which the interval between the first and last mating was the shortest (1–4 days) and the longest (9 days and above) (Table 3). The significantly lower reproductive parameters of the females coupled with the considerable spread between the first and last mating compared to mink mated on 2, 3 and 4 consecutive days confirm the claim that delayed embryo implantation in the uterus has an adverse effect on embryo development (Berglund, 1986; Sundqvist et al., 1989). This indicates that the large interval between the first and last mating is unfavourable, especially because the embryos generated after first mating may die (Sundqvist et al., 1989). The risk of blastocyst loss increases with the increasing period from fertilization to implantation (Møller, 1997). In addition, Isakova (2007), who studied the karyotypes of monochorionic twins in the postimplantational embryogenesis of mink, found the presence of different chromosome sets (2n, XX and 2n, XY or 2n and 3n). This fact contradicts the idea that monochorionic twins should be genetically identical but confirms the hypothesis that a third twinning type exists in mink: monozygotic but genetically different. It is suggested that the high frequency of this phenomenon in the mink is related to embryonic diapause, causing abnormal fertilization. These findings justify our results, which show that reproductive performance of mink does not improve when the first and last matings are several days apart.

Of course another explanation for these results is possible. The mating of females to different males triggers mechanisms that reduce the likelihood of fertilization by the semen of a genetically different male. Under this assumption embryos from fertilization during the first mating survive, while the next mating results in no fertilization because there are genetic or environmental factors that prevent the fertilization. One example is the seminal fluid of *Drosophila melanogaster* males, which increases their siring success relative to other males while shortening the lifespan of females (Chapman et al., 2003). Many male adaptations associated with sperm competition that enable the male to achieve reproductive success have been described. For example, males can remove rival sperm mechanically or chemically, which affects evolution of the morphology of genital organs or seminal fluids (Birkhead and Moller, 1998).

Both theories discussed above are probable, but the available literature contains few publications that confirm or exclude them, because both justify the results obtained in the present study. The relationships mentioned above could only be validated using molecular data that determine the proportions of kits sired by different fathers. Thus, the research should be continued at the molecular level. Multiple paternity in free-ranging mink was confirmed using 7 molecular markers (Yamaguchi et al., 2004). Studies with a population of breeding raccoon dogs (Ślaska and Jeżewska, 2008), which like mink are members of the order *Carnivora*, also confirmed the phenomenon of multiple paternity when females were mated to different males during one oestrus. Multiple paternity was also described in the European badger (*Meles meles*) (Dugdale et al., 2007), which like the mink belongs to the *Mustelidae* family. It is possible that multiple paternity may be one of significant factors affecting reproductive results in animals. The use of molecular techniques would enable determining which theory of embryonic development in the mink is correct, and this indicates the need for continued research in this area.

The short interval between consecutive matings may ensure that the diapause period is short. It can thus cause rapid implantation of embryos in the uterus. Our research and the literature cited above suggest that this may increase the level of reproductive performance in the mink. This conclusion follows from the analyses indicating that female reproductive parameters were significantly the best when the mating period was short. The considerable interval between the first and last mating may be particularly disadvantageous because it increases the time until embryo implantation in the uterus.

In summary, we can say that the level of reproductive performance in mink was dependent on the mating system, in particular the lack of diapause or the presence of several-day diapause between successive matings. Regardless of the number of matings of the female, the most favourable level of reproductive performance was characteristic of the mink in which the interval between the first and last mating was the shortest (1–4 days). When the matings were much apart (9 days and above), reproductive parameters of the females were significantly lower. When choosing the mating system, care must be taken to ensure that it does not prolong the diapause period, which at the same time should reduce the losses associated with embryo death and resorption. In addition, the lack of significant differences for reproductive parameters of the females mated on 2, 3 and 4 consecutive days indicates that mating mink more than twice (with the 1+1 mating system but not 1+9) has no economic justification and only increases labour inputs.

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SYSTEM KOJARZEŃ A POZIOM CECH REPRODUKCYJNYCH NOREK (*NEOVISON VISON*)

STRESZCZENIE

Celem pracy była odpowiedź na pytanie w jakim stopniu system krycia samic norek warunkuje poziom cech reprodukcyjnych tego gatunku, czy jest to czynnik, który w istotny sposób determinuje liczebność miotów, a jeżeli tak, to który ze stosowanych na fermach systemów można uznać za optymalny. Obserwacjami objęto 1626 norek stada podstawowego. Przeanalizowano siedem różnych systemów różniących się liczbą kryć i odstępem pomiędzy kolejnymi kryciami samicy. Wpływ systemów krycia na poziom cech reprodukcyjnych weryfikowano wieloczynnikową analizą wariancji metodą najmniejszych kwadratów. System krycia definiujący w tym przypadku zarówno liczbę stosowanych kryć jak i liczbę dni pomiędzy nimi okazał się czynnikiem w wysoko istotny sposób determinującym liczebność miotów. Bez względu na liczbę kryć samic, najkorzystniejszym poziomem cech reprodukcyjnych charakteryzowały się norki, u których rozpiętość pomiędzy pierwszym i ostatnim kryciem była najmniejsza. Przy wyborze systemu należy zwrócić uwagę na to, aby nie przedłużał on okresu diapauzy, co jednocześnie powinno wpłynąć na zmniejszenie strat wynikających z zamierania i resorpcji zarodków.