

MULTIPLE PATERNITY IN AMERICAN MINK: USING MALES OF DIFFERENT COLOR LINES ALLOWS MATING EFFICIENCY EVALUATION

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ABSTRACT

The study aimed at developing an optimal mink (*Neovison vison*) mating system by examining the effectiveness of multiple paternity, which was achieved by mating a female to two males of contrastingly different color lines over an interval of time within the same estrus. The hypothesis tested was that embryos generated from the first mating survived and developed despite subsequent matings. The experiment was carried out in two consecutive breeding seasons on a mink farm. We used pure genetic lines of the coat-color varieties. In order to find the most effective system of mating, we counted the offspring sired by each male. Mating to two males of different coat colors on two, time-separated dates produced litters consisting of two distinctly marked groups of kits, easily recognizable as to which kit had been sired by which male. This suggests that blastocysts generated from the first mating do survive until parturition and, what is more, develop normally. Despite prior mating to another male, kits derived from the subsequent mating were more numerous in the litter.

Key words: American mink, breeding, multiple mating

INTRODUCTION

The life history of the American mink (*Neovison vison*) is characteristic for the complexity of the reproductive process. This may be a challenge for the farmer; if the breeding performance of a given season is not satisfying, the complexity of the mink reproductive biology does not facilitate diagnosis of the problem. The physiological specificity is marked by monoestrous character, delayed blastocyst implantation, and—in consequence—a varying length of gestation [Yamaguchi et al. 2004, Thom et al. 2004].

Diapause, which is followed by photoperiod-triggered resumption of embryonic development and implantation, allows the embryos derived from several joinings which occur on different days to level their development before birth. As a result, total gestation length may vary greatly, from 36 to 85 days; however, most often gestation remains in the range 45 to 55 days [Tauson

et al. 2000, Persson 2007, Seremak et al. 2009, Felska-Błaszczuk et al. 2010]. During diapause, the development of the embryo is reversibly restrained at the stage of blastocyst and the process of its implantation in the uterus is delayed [Desmarais et al. 2004, Lopes et al. 2003, Lefèvre et al. 2011]. Diapause in mink also varies in length, depending on the moment of implantation [Wehrenberg et al. 1992, Polejaeva et al. 1997, Lopes et al. 2003]. According to Lefèvre et al. [2011], diapause lasts 2–3 weeks on average, although it is possible to extend to more than three months [Rose et al. 1986, Martinet et al. 1981]. Reactivation from diapause is triggered by the increasing photoperiod following the vernal equinox, which results in an increase in circulating prolactin and a subsequent increase in ovarian progesterone synthesis [Renfree and Fenelon 2017, Tauson et al. 2000, Lopes et al. 2004]. According to Murphy and James [1974], the daylight phase of 12 to 16 hours is an

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optional signal to induce implantation. Estrus is initiated when the daylight phase reaches 10 hours, i.e. when the day becomes about two hours longer than the eight-hour winter day [Klotchkov et al. 1998, Felska-Błaszczuk et al. 2010]. The period between implantation and birth, 30–31 days, is rather constant [Song et al. 1998, Gulevich et al. 1995, Polejaeva et al. 1997, Tauson et al. 2000, Klotchkov and Eryuchenkov 2003].

Sexual maturity is attained at age 8–12 months and is characterized by recurring morphological and hormonal changes in the reproductive system linked with the estrus, i.e. folliculogenesis, ovulation, luteinization and luteolysis of the corpus luteum. Cyclic maturation of oocytes over time allows multiple matings without breaking the estrus, which is a characteristic of the species [Persson 2007].

Typically, the mink sustain sexual activity for a relatively short period of time [Travis and Pilbeam 1980] and a breeding season lasts 2–3 weeks [Trani et al. 2007, Seremak et al. 2009] or 1 month at most [Klotchkov and Eryuchenkov 2003]. Follicles continuously mature on the ovaries during the season of sexual activity, with their greatest numbers appearing – in the moderate climate of Poland – between March 10 and 20. The continuous maturation of ova during the estrus period is addressed with a system of mating a single female to one or more males at several times [Seremak et al. 2009]. Copulation, ovulation or conception do not shorten the period of estrus [Persson 2007], and blastocysts resulting from the initial mating develop, despite any subsequent copulations [Murphy 1983, Polejaeva et al. 1997, Klotchkov and Eryuchenkov 2003].

Many farms apply a group system of breeding in which a group of closely related females is joined with an unrelated pool of males which are also related to each other. Such a mating system allows changing the sires while maintaining genetic improvement of the herd [Seremak et al. 2011]. Many farmers are concerned with selecting the most suitable date to start breeding in order to obtain the greatest conception rates and largest litters. Long-established on-farm practice and observations have allowed the farmers to develop mating systems that are appropriate for this particular species. Farm practices include double mating, according to the formula 1 + 8 or 1 + 9 (numbers represent subsequent days on which mating takes place), triple mating, 1 + 2 + 8, 1 + 2 + 9 or 1 + 8 + 9, and quadruple mating, according to the formula 1 + 2 + 8 + 9 [Ślaska and Rozempolska-Rucińska 2011]. However, on some farms females are still mated only once during the entire reproductive season.

The decision to choose the best mating system is crucial to obtaining large litters. Hence, this study was conducted to check the effectiveness of multiple paternity by joining females with males so that the offspring of each mating would differ in coat color. The hypothesis tested

assumed that the embryos generated from the first mating survived and developed despite the subsequent mating.

MATERIAL AND METHODS

This study was carried out in strict accordance with the recommendations of the Polish Act dated 21 January 2005 on Animal Experiments (Journal of Laws 2005, no. 33, pos. 289). The protocol was approved by the Local Ethical Committee for Experiments on Animals at the West Pomeranian University of Technology, Szczecin, Poland (permit number: 3/2013, dated 7 March 2013).

The experiment was carried out during two consecutive years (breeding seasons) on a farm located in northern Poland. The mink were managed in an open-shed system, fed a standard semiliquid chicken- and fish-based feed, according to the standards for the species [PAN 2011]. During the breeding season, the mink were arranged in 70 breeding sets. Each breeding set consisted of a group of 40 females, divided into 5 sections of 8 females each, and 8 males in a sixth section. On the selected day, in the morning, females of a section were monogamously mated to one of the males. Another section of females was similarly mated that evening. Thus, each male had potentially served two females daily. On the following day, another section of females was mated to the males, and so forth.

The studied herd consisted of 2738 females (1791 Pearl, P, and 947 White Hedlund, WH) and 548 males (178 Pearl, 94 White Hedlund, 180 White Regal, WR, and 96 Black Cross, BC), all the animals at age 1 year. Pure genetic lines of color variants were used in order to find the proportion of offspring sired by each male in the litter of a multiple-mated female. Table 1 presents coat colors of the progeny obtained from crossing breeders of each color variant.

Table 1. Coat colors of the kits born from crossing applied color lines

Tabela 1. Kolory urodzonych młodych z poszczególnych krzyżowań odmian barwnych rodziców

Male color Odmiana barwna samca	Female color Odmiana barwna samicy	
	Pearl Perła	White Hedlund Biała Hedlunda
Pearl Perła	Pearl Perła	Pastel Pastel
White Hedlund Biała Hedlunda	Pastel Pastel	White Hedlund Biała Hedlunda
White Regal Biała Regal	Pastel Pastel	–
Black Cross Czarny Krzyżak	–	Black Cross Czarny Kryżak

Table 2 presents the scheme of matings in both years of the experiment. The first mating date was assumed as the day the female was bred for the first time in the season (not later than March 7) with a repetition the next day (if any). The second mating took place after seven days and was also possibly repeated the next day, as well as a single mating (with or without repetition the next day) performed later than March 7. All the data were obtained through non-invasive observation which did not require contact with the mink and were carried out during normal farm operations.

The following mating patterns were designed in relation to multiplicity and dates of matings (Arabic digits, 1 or 2, denote the number of matings, whereas Roman numerals, I or II, denote the date of mating):

- 1-I – single mating on the first date
- 1-II – single mating on the second date
- 2-I – double mating on the first date
- 2-II – double mating on the second date
- 1-I 1-II – single mating on the first date and single mating on the second date
- 1-I 2-II – single mating on the first date and double mating on the second date
- 2-I 1-II – double mating on the first date and single mating on the second date
- 2-I 2-II – double mating on the first date and double mating on the second date

The mating process was supervised by designated farm workers, each copulation was duly registered in the female's records.

Statistical analyses

Data on the number of kits born from each mating were analyzed statistically using the Statistica 12PL package. Descriptive statistics included the arithmetic mean, *m*, and standard error, *SE*. HSD Tukey test and two-way ANOVA were performed to test the significance of differences between the values. For fertility, we used the non-parametric Kruskal-Wallis test to compare many samples of independent groups.

RESULTS

The results presented in Tables 3 and 4 are not broken by the color variants of males and females, since we did not analyze the information on the reproduction performance of particular variants. Using pure genetic lines of each color variant was aimed entirely to mark the offspring produced in the given mating scheme.

Fertility of Pearl dams the first year of the study are presented in Table 3. The most numerous litters, 7.27 born and 6.58 live-born kits, were attained by females

mated four times (2-I 2-II) during the breeding season. These differed significantly (at $P < 0.01$) from the litter sizes produced by dams mated once, i.e. on the first date (1-I, 5.07 kits), or on the second date (1-II, 6.11 kits), or mated twice on the first date (2-I, 6.18 kits, and 2-II, 6.14 kits). The lowest average litter of live-born kits, 4.56 individuals, was observed in the group of females mated once on the first date (1-I). If we look at the litter size sired by the first and the second male with the same number of matings (two mating schemes, 1-I 1-II, and 2-I 2-II), more kits may be born if either a single or double mating be repeated using another male. The mean litter sizes obtained from these schemes were, respectively, 3.9 and 5.79, while for the same matings with the first male, 2.80 and 1.48 kits were born only, respectively.

A similar relationship was observed comparing two systems of triple mating according to the scheme 1-I 2-II (single mating in the first and double in the second term) and 2-I 1-II (double mating in the first and single one in the second term), for which the average litter size was 1.21 and 1.63, on the first date, and 5.69 and 5.14 kits, on the second date. It is worth noting that in the 2-I 1-II system a higher average, 5.14 kits, was obtained from the single mating in the second term, compared to the average, 1.63, obtained from the double mating on the first date.

Analysis of White Hedlund females breeding in year 2 (Table 4) reveals that the largest litters were produced by dams mated four times, i.e. twice on the first and twice on the second date (scheme 2-I 2-II) with 7.36 kits per litter on average, significantly ($P < 0.01$) higher in relation to those mated once or twice on the first date, which produced 6.11 and 5.44 kits per litter, respectively (Table 4). Those dams also produced the largest live-born litters, 6.98 kits on average. By far the lowest litter sizes of born (5.44) and live-born kits (5.05) were obtained from females mated once on the first date of the breeding season. These data differed significantly ($P < 0.01$) between litters produced by dams mated in 1-II and 2-II schemes, i.e. 7.46 and 7.18, respectively, born and live-born kits per litter, and those obtained from females mated in systems 2-I and 2-1 2-II, which averaged 5.58 and 6.98 kits per litter, respectively. The average live-born litter size (5.05 kits) in the dams mated 1-I differed significantly ($P < 0.05$) from that obtained from dams mated three times 1-I 2-II.

DISCUSSION

The American mink breeding physiology is characteristic for superfetation, or fertilization of oocytes ovulating in subsequent cycles, which leads to implantation of another ovum without shortening the oestrus cycle [Persson 2007]. Another specificity is superfecundation, i.e. fertilization of the egg cells generated within a single ovulation cycle by sperm coming from different, time-

Table 2. Mating scheme by year of experiment; color variants of females and of males on the first and second date

Tabela 2. Krycia w kolejnych latach badań; odmiany barwne samic i samców dopuszczonych do kryć w pierwszym i drugim terminie kojarzeń

	Female color variant Odmiana barwna samicy	Male color variant Odmiana barwna samca	
		First mating date, I Krycie w pierwszym terminie, I	Second mating date, II Krycie w drugim terminie, II
		First year Pierwszy rok	P
	P	WR	P
Second year Drugi rok	WH	WH	BC
	WH	BC	WH

Table 3. Fertility of mink as the average litter size obtained from mating dates I and II, by date and the number of matings in the first year

Tabela 3. Plenność samic norek z uwzględnieniem średniej liczby norczą uzyskanych z I oraz II terminu kojarzenia w zależności od zastosowanych terminów i krotności kryć w pierwszym roku badań

Pattern of mating Krotność oraz termin kryć	Number of matings Liczba kryć	N	Litter size overall Liczba urodzonych młodych w miocie		No. of kits derived from each mating (mean) Liczba młodych z poszczególnych kryć (średnia)		No. of live-born kits per litter Liczba żywo urodzonych w miocie	
			m	SE	I date I termin	II date II termin	m	SE
			1-I	1	57	5.07 ^{A^{EF}G}	0.38	5.07
1-II	1	37	6.11 ^B	0.44	–	6.11	5.43	0.41
2-I	2	228	6.18 ^C	0.20	6.18	–	5.14 ^{BC}	0.19
2-II	2	127	6.14 ^D	0.24	–	6.14	5.70	0.24
1-I 1-II	2	81	6.70 ^E	0.26	2.80 ^{**}	3.90 ^{**}	6.01	0.25
1-I 2-II	3	172	6.90 ^F	0.19	1.21 ^{**}	5.69 ^{**}	6.29 ^{AB}	0.19
2-I 1-II	3	135	6.77 ^G	0.23	1.63 ^{**}	5.14 ^{**}	5.95 ^a	0.23
2-I 2-II	4	628	7.27 ^{ABCD}	0.08	1.48 ^{**}	5.79 ^{**}	6.58 ^{AC}	0.09

A, B, C... means in columns marked with the same letters differ significantly at $P \leq 0.01$ (upper case) and at $P \leq 0.05$ (lower case letters);

**means in rows differ significantly at $P \leq 0.01$.

A, B, C... średnie w kolumnach oznaczone tymi samymi literami różnią się istotnie przy $P \leq 0,01$, a małymi literami przy $P \leq 0,05$; **średnie w wierszach różnią się statystycznie przy $P \leq 0,01$.

separated sexual acts [Lefèvre and Murphy 2008]. Thus, the cyclic maturation of oocytes per heat allowed the development of a multiple-mating system using the same or different males [Seremak et al. 2009]. Both our results and those reported by Ślaska et al. [2009] confirm that the litter sizes in farmed mink are larger if more matings are applied.

Besides the outcomes of multiple matings, our study shows that the date of mating is important for the quality of reproduction parameters. Although the latter proves crucial in terms of breeding performance, it is often neglected in the subject literature or discussed only in general. The impact of the date of mating was evaluated by means of the mean size of the litter sired by males of dif-

ferent coat colors (light or dark). This was possible since we applied crossing males and females of pure genetic lines of contrasting color variants.

The positive effect of multiple mating for litter size has also been reported by Møller [1974], who assessed this gain at a level of 0.2–0.3 kits per litter. Likewise, Ślaska et al. [2009] attained a higher level of fertility in mink females mated repeatedly as compared to those mated only once or twice. The multiple matings should not be, however, considerably extended in time, as this makes the diapause longer, which is adverse in relation to embryonic survival rates [Rozempolska-Rucińska et al. 2004]. This is linked with the fact of ovulation recurring several times during a breeding season and the possibil-

Table 4. Fertility of mink as the average litter size obtained from mating dates I and II, by date and the number of matings in the second year

Tabela 4. Plenność samic norek z uwzględnieniem średniej liczby norczą uzyskanych z I oraz II terminu kojarzenia w zależności od zastosowanych terminów i krotności kryć w drugim roku badań

Pattern of mating Krotność oraz termin kryć	Number of matings Liczba kryć	N	Litter size overall Liczba urodzonych młodych w miocie		No. of kits derived from each mating (mean) Liczba młodych z poszczególnych kryć (średnia)		No. of live-born kits per litter Liczba żywo urodzonych w miocie	
			m	SE	I date I termin	II date II termin	m	SE
1-I	1	39	5.44 ^{ACDEFa}	0.44	5.44	–	5.05 ^{ABCDH}	0.46
1-II	1	122	7.16 ^C	0.21	–	7.16	6.18	0.23
2-I	2	88	6.11 ^B	0.28	6.11	–	5.58 ^{EFG}	0.27
2-II	2	164	7.18 ^D	0.18	–	7.18	6.57 ^P	0.19
1-I 1-II	2	38	7.00 ^E	0.44	1.67 ^{**}	5.33 ^{**}	6.71 ^{CG}	0.41
1-I 2-II	3	77	7.25 ^F	0.24	0.83 ^{**}	6.42 ^{**}	6.88 ^{BF}	0.24
2-I 1-II	3	109	6.89 ^a	0.23	0.49 ^{**}	6.40 ^{**}	6.44 ^H	0.23
2-I 2-II	4	175	7.36 ^{AB}	0.14	2.10 ^{**}	5.26 ^{**}	6.98 ^{AE}	0.15

A, B, C... means in columns marked with the same letters differ significantly at $P \leq 0.01$ (upper case) and at $P \leq 0.05$ (lower case letters); **means in rows differ significantly at $P \leq 0.01$.

A, B, C... średnie w kolumnach oznaczone tymi samymi literami różnią się istotnie przy $P \leq 0,01$, a małymi literami przy $P \leq 0,05$; **średnie w wierszach różnią się statystycznie przy $P \leq 0,01$.

ity to produce offspring from time-separated ovulations [Sundqvist et al. 1989].

The fertilization of an already pregnant female in subsequent ovulations is possible due to delayed implantation and embryonic diapause [Yamaguchi et al. 2006], which in turn is associated with a low level of progesterone produced by corpora lutea, insufficient to prevent subsequent ovulations. Applying a 7-day break and mating on day 8 is reasonable, as supported by Elofson et al. [1989], and allows another ovulation to come forth. From the perspective of the species reproduction physiology, such a system of mating is indeed fully justified.

Our results confirm the findings reported by Polejaeva et al. [1997] and Klotchkov and Eryuchenkov [2003], who claim that blastocysts from the first mating develop despite other subsequent matings. The results presented in this study show, however, that the largest number of kits in a litter originate from the second mating, which implies that many embryos from the first mating probably die during the diapause. Some portion of them, however, do survive until birth, positively contributing to the resulting litter size. Delayed implantation in mink probably plays the key role in the litter size regulation. Extended period of diapause increases embryonic mortality, and in extreme may lead to complete die out of all the embryos, which may be mistakenly taken as female barrenness. Multiple paternity in mink is not an isolated phenomenon among animals, similar effect has been ob-

served in raccoon dogs [Ślaska and Jeżewska 2008] and badgers [Dugdale et al. 2007].

Ślaska et al. [2009] confirm the observation that the number of matings is positively correlated with litter size in mink. We found that, besides the multiplicity of mating, the breeding outcomes also depend on the date of mating. The latter proved to be very significant in terms of reproduction parameters, yet the literature seems to avoid this topic or treats it without a deeper reflection. Its efficiency was tested on the average number of kits derived from different sires of contrasting (light or dark) coat colors. This was possible by using pure genetic lines of various color varieties of males and females. The results confirm data reported by Polejaeva et al. [1997] and Klotchkov and Eryuchenkov [2003], who claim that blastocysts originating from the first mating do develop despite subsequent multiple copulations. Our studies show, however, a dominance of the kits derived from the second mating, which may imply that a number of embryos from the previous conception probably die during the diapause. Some of them, though, survive and enlarge the total size of the litter. The phenomenon of delayed implantation in mink is likely to play a key role in the sizes of litters. An extended period of diapause promotes the mortality of embryos; should all of them die, this may be mistakenly taken as female sterility.

CONCLUSION

The results obtained in both the first and the second year of the experiment confirm the significance of timing and multiplicity of mating during the breeding period and confirm the desirability of mating American mink females in two dates (breeding both in the first and second date) of the breeding season. Breeding females to males of two different colour varieties on two time-separated dates produced litters consisting of two groups of kits, light and dark in color. This suggests that blastocysts derived from the first mating survive and develop, despite subsequent multiple matings to other males; kits derived from the second breeding, however, predominate in the litter.

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WIELOOJCOSTWO U NORKI AMERYKAŃSKIEJ: ZASTOSOWANIE SAMCÓW RÓŻNYCH ODMIAN BARWNYCH POZWALA OCENIĆ EFEKTYWNOŚĆ KRYCIA

STRESZCZENIE

Badanie miało na celu opracowanie optymalnego systemu kojarzenia norek (*Neovison vison*) poprzez zbadanie skuteczności wielokrotnego ojcostwa, które zostało osiągnięte poprzez skojarzenie samicy z dwoma samcami o kontrastująco różnych kolorach w pewnym okresie w tej samej rui. Testowano hipotezę, że zarodki powstałe podczas pierwszego krycia przeżyły i rozwinęły się pomimo kolejnych kryć. Doświadczenie prowadzono w dwóch kolejnych sezonach rozrodczych na fermie norek. Do badań wykorzystano czyste linie genetyczne odmian barwnych. W celu znalezienia najbardziej efektywnego systemu kojarzenia, policzyliśmy potomstwo pochodzące od każdego z samców. Kojarzenie z dwoma samcami o różnych umaszczeniach w dwóch oddzielonych w czasie terminach skutkowało w postaci miotów składających się z dwóch wyraźnie odróżniających się grup norcząt. Sugeruje to, że blastocysty powstałe z pierwszego krycia przeżywają do porodu, a ponadto rozwijają się normalnie. Pomimo wcześniejszego krycia z udziałem innego samca, w miocie liczniejsze były młode pochodzące z kolejnego krycia.

Słowa kluczowe: norka amerykańska, reprodukcja, krycie wielokrotne