

## MATING SYSTEM VS. LITTER SIZE IN FARM MINK (*NEOVISON VISON*) – EFFECT OF MULTIPLE PATERNITY

Lidia Felska-Błaszczyk<sup>1</sup> , Beata Seremak<sup>2</sup> , Natalia Ławrów<sup>1</sup>

<sup>1</sup>Laboratory of Animal Anatomy, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology in Szczecin, Doktora Judyma 14, 71-466 Szczecin, Poland;

<sup>2</sup>Department of Biotechnology of Animal Reproduction and Environmental Hygiene, Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology Szczecin, Klemensa Janickiego 29, 71-270 Szczecin, Poland

### ABSTRACT

The aim of the study was to analyze various mating systems, mating dates, and the number of matings applied for farmed American mink in relation to resulting litter sizes. The observations were carried out on yearling females of two color varieties, Perl (P) and White Hedlund (WH), as well as yearling males belonging to four color varieties, namely P, WH, White Regal (WR) and Black Cross (BC). The color of the males was chosen so as to easily discern, which male sired which litter and what was the litter size resulting from the particular copulation date. The breeders were mated according to various mating schemes. The analysis revealed that if two matings were the case, most embryos conceived on the first date died.

**Key words:** reproduction, superfetation, system of mating

### INTRODUCTION

The mink is a monoestrous species with the heat occurring in March under the moderate climate, such as the one in Poland. There are 2 to 3 ovarian cell maturation cycles per heat, and an effective fertilization of ovulated cells resulting from the first mating may be followed by another ovulation and conception, which implies that the female may be mated to different males a number of times within a single sexual activity season. The effect of ovulation, fertilization and implantation of the second set of eggs within the same pregnancy is called superfetation [Roelling et al. 2011]. Superfetation is connected with superfecundation, also known as polyspermia, which consist in fertilizing in subsequent mating encounters oocytes produced in the same heat with sperm from different males, so that offspring born in the same litter may have been sired by different males. This is referred to as multiple paternity [Thom et al. 2004, García 2010, Macdonald et al. 2015], which is an adaptation allowing greatest possible reproductive success. Superfetation in mink is favoured by diapause, the period of delayed implantation of embryos [Macdonald and Harrington 2003].

Multiple paternity in wild American mink populations has been studied by Yamaguchi et al. [2004] and Thom et al. [2004], who observed that American mink males abandon the instinct of territoriality during the heat, and each female mates with several males, on average 11.75 times during the mating season. Such a large number of possible copulations within one sexual activity season has also been observed in farmed mink; Seremak et al. [2013] found that females placed in males' cages every day during the heat were very active sexually and copulated as many as 8–9 times during this period, which confirms the findings of Thom et al. [2004].

Both intensive farming systems and the biological specificity of mink reproduction (superfetation and superfecundation) make farmers seek best methods and systems which would allow obtaining large and strong litters. The most important factors affecting the reproduction performance of mink include the color variety and age of the animals; however, the breeding season operations, such as planning the timing of mating, system and number of applied matings, are crucial in this respect as well.

The aim of the study was to analyze various systems and dates of mating as well as the number of matings in

✉ lidia.felska-blaszczyk@zut.edu.pl

relation to litter size, and sex distribution in the litter of mink in relation to various factors.

## MATERIAL AND METHODS

The studies were carried out on a mink farm located in the northern part of Poland. The animals were managed in an open-shed system, fed a standard semiliquid feed based on chicken and fish. The material consisted of (Table 1):

- One year old mink females of two color varieties, Pearl (P) and White Hedlund (WH),
- One year old mink males of four color varieties, Pearl (P), White Hedlund (WH), White Regal (WR), and Black Cross (BC).

The colors of the male minks were selected so that it would be easily determine which male sired which offspring and, in consequence, how many kits were born as a result of mating on a particular day.

Pearl females which on the first date were mated to Pearl males gave birth to Pearl offspring; however, mated seven days later to White Regal males, the same females gave birth to Pastel (Ps) kits. Other Pearl females, mated to White Regal males on the first date, gave birth to Pastel offspring; however, the same Pearl females, mated seven days later to Pearl males, produced offspring of the Pearl variety.

White Hedlund females that were mated to White Hedlund males on the first date gave birth to White Hedlund offspring; however, mated seven days later to Black Cross males, the same females gave birth to Black Cross kits. Other White Hedlund females, mated to White Regal males on the first date, gave birth to Pastel offspring; however, the same White Hedlund females, mated seven days later to White Hedlund males, produced White Hedlund offspring.

The animals of the breeding stock were mated according to the following schemes (an Arabic digit denotes how many times females were mated, while a Roman numeral is the date of the 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

- 1–I 3–II: single mating on the first date and triple mating on the second date

We analyzed litter size at birth and live-births per litter in relation to the color variety of the female and the male, as well as the mating system.

The statistical calculations and analysis was performed using the STATISTICA 13.3 PL package. The statistical analysis was carried out using a oneway analysis of variance (ANOVA). The significance of differences between mean values of traits was calculated with the Duncan multiple range test. Statistical description involved the mean (m), standard deviation (SD).

## RESULTS

If we look at the Pearl females mated to Pearl or White Regal males (Table 2), the group of females mated four times (2–I 2–II) within the breeding season were characterized by the highest fertility, with an average litter size of 7.20 (6.61 live-born) kits.

These results differ significantly (at  $P \leq 0.01$ ) from those obtained for total litter sizes of females mated twice on the first date (2–I), 6.14 kits, and twice on the second date (2–II), 6.16 kits, but also in relation to an average number of life-born kits per litter in females mated twice on the first date (2–I), 5.16 kits. The lowest average litter sizes of born (5.05) and live-born (4.45) kits were obtained after a single mating on the first date (1–I). These results differ significantly (at  $P \leq 0.01$ , for born, and  $P \leq 0.05$ , for the live-born kits) from the highest values obtained for females mated four or three times (2–I 2–II, 2–I 1–II, 1–I 2–II) during the breeding season.

Table 2 also presents the results regarding the size of the litter depending on the mating date. The effectiveness of the mating date was assessed on the basis of the average number of kits (light and dark) born after mating to a male of the selected color variant, with which females were paired in the given period. Analyzing the size of litters obtained from a Pearl (P) female depending on the date of mating (dates I and II), when females were mated the same number of times both in the first and second term (these are systems 1–I 1–II and 2–I 2–II), it was observed that more kits were born after mating on the second date, respectively 3.91 and 5.79 individuals in the litter. However, for the same females mated on the first date a lower number of born kits was obtained, 2.80 and 1.41 kits on average. It can be concluded that in all the cases when females were mated on both the first and the second date, regardless of the number of matings, much larger litters were born after the matings on the second date and this difference was significant at  $P \leq 0.01$  and  $P \leq 0.05$ .

**Table 1.** Number and color variety of treatment groups of females with respect to the color of males mated on dates I and II

**Tabela 1.** Liczba oraz odmiana barwna samic norek grup doświadczalnych z uwzględnieniem odmiany barwnej samców norek kryjących w I i II terminie

Color variants of females Odmiana barwna samicy	Number of females Liczba samic	1st mating (I), March 3 to March 10 Pierwsze krycie (I), od 3 do 10 marca		2nd mating (II), March 11 to March 18 Drugie krycie (II), od 11 do 18 marca	
		Color variant of males Odmiana barwna samców			
Pearl Perła	950	Pearl Perła		White Regal Biały regal	
Pearl Perła	940	White Regal Biały regal		Pearl Perła	
White Hedlund Biała Hedlunda	550	White Hedlund Biały Hedlunda		Black Cross Czarny krzyżak	
White Hedlund Biała Hedlunda	570	Black Cross Czarny krzyżak		White Hedlund Biały Hedlunda	
Total Razem	3010				

**Table 2.** Results of mating Pear females to Pearl or White Regal males

**Tabela 2.** Wyniki kojarzenia samic odmiany perła z samcami odmiany perła lub biała regal

Number and date of mating Liczba i data krycia	Number of matings Liczba kryć	Number of females Liczba samic	Litter size Wielkość miotu		Litter size in relation to date of mating Wielkość miotu w odniesieniu do daty krycia		Live-born per litter Liczba żywo urodzonych młodych w miocie	
			$\bar{x}$	SD	I date I data	II date II data	$\bar{x}$	SD
1-I	1	118	5.05 <sup>A</sup>	2.81	5.05	–	4.45 <sup>Aa</sup>	2.84
1-II	1	110	6.11	2.72	–	6.11	5.43	2.52
2-I	2	252	6.14 <sup>B</sup>	3.03	6.14	–	5.16 <sup>BC</sup>	2.88
2-II	2	200	6.18 <sup>C</sup>	2.70	–	6.18	5.70	2.74
1-I 1-II	2	120	6.70	2.28	2.80*	3.91*	6.01	2.30
1-I 2-II	3	268	6.91 <sup>A</sup>	2.49	1.21**	5.69**	6.29 <sup>AB</sup>	2.48
2-I 1-II	3	154	6.75 <sup>A</sup>	2.63	1.61**	5.14**	5.90 <sup>a</sup>	2.65
2-I 2-II	4	512	7.20 <sup>ABC</sup>	2.17	1.41**	5.79**	6.61 <sup>AC</sup>	2.29
1-I 3-II	4	156	6.73	2.08	0.76**	5.97**	6.22	2.14

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

A, B, C... – średnie w kolumnach oznaczone tymi samymi literami różnią się istotnie przy  $P \leq 0,01$  (wielkie) lub przy  $P \leq 0,05$  (małe litery).

\*\* – means in rows differ significantly at  $P \leq 0.01$  (\*\*\*) or  $P \leq 0.05$  (\*).

\*\* – średnie w wierszach różnią się statystycznie przy  $P \leq 0,01$  (\*\*\*) przy  $P \leq 0,05$  (\*).

The analysis of breeding results obtained for the WH females (Table 3) showed the highest average number of born offspring at the level of 7.43 for females mated once in the second term of the breeding season. This scheme of mating, however, turned out to be disadvantageous due to the fact that there was a significant difference between the average number of born and live born offspring. On the basis of the following results, similarly as in the fertility studies, also the analysis of the dates and number of matings of White Hedlund females confirmed the predominance of four matings – twice on the first and twice on the second date of the breeding season (2-I 2-II). The average number of born kits in the litter, 7.34, for females mated according to the above scheme, was statistically (at

$P \leq 0.01$ ) higher in relation to the results obtained for a group of dams mated only once or twice on the first date, in which these values were 5.42 and 6.09 in the litter, respectively. In the group of WH females mated four times (scheme 2-I 2-II), also the average number of live-born kits was highest, 6.98 kits in the litter. By far the poorest results were attained by the females mated only once on the first date, that is 5.42 born (5.05 live-born) kits per litter.

Again, also in the White Hedlund variety females, the analysis of the mating schemes showed that the second mating date proved to be the most advantageous due to the higher values of the reproduction parameters.

**Table 3.** Results of mating White Hedlund females to White Hedlund or Black Cross males

**Tabela 3.** Wyniki kojarzenia samic odmiany białą Hedlunda z samcami odmiany białą Hedlunda lub czarny krzyżak

Number and date of mating Liczba i data krycia	Number of matings Liczba kryć	Number of females Liczba samic	Litter size Wielkość miotu		Litter size in relation to date of mating Wielkość miotu w odniesieniu do daty krycia		Live-born per litter Liczba żywo urodzonych młodych w miocie	
			$\bar{x}$	SD	I date I data	II date II data	$\bar{x}$	SD
1-I	1	107	5.42 <sup>ACa</sup>	2.74	5.42	–	5.05 <sup>Aa</sup>	2.86
1-II	1	150	7.43 <sup>AB</sup>	2.35	–	7.43	6.45	2.53
2-I	2	128	6.09 <sup>BC</sup>	2.58	6.09	–	5.58 <sup>AB</sup>	2.52
2-II	2	164	7.18 <sup>A</sup>	2.33	–	7.18	6.57	2.47
1-I 1-II	2	124	7.00	2.71	1.67**	5.33**	6.71	2.52
1-I 2-II	3	112	7.25 <sup>a</sup>	2.08	0.83**	6.42**	6.88 <sup>Ba</sup>	2.15
2-I 1-II	3	150	6.84	2.29	0.49**	6.36**	6.44	2.39
2-I 2-II	4	185	7.34 <sup>C</sup>	1.82	2.09**	5.25**	6.98 <sup>A</sup>	1.96

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

A, B, C... – średnie w kolumnach oznaczone tymi samymi literami różnią się istotnie przy  $P \leq 0,01$  (wielkie) lub przy  $P \leq 0,05$  (małe litery).

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

\*\* – średnie w wierszach różnią się statystycznie przy  $P \leq 0,01$ .

## DISCUSSION

Statistically significant differences in the reproduction performance, resulting from a varying number of matings, have been reported by Felska-Błaszczczyk [2012] and Felska-Błaszczczyk et al. [2012]. The authors state that applying a single-mating system of breeding returns a lower average litter size. The largest litters, on the other hand, were attained by females mated four times during the heat. The fact that mating breeding mink in two dates is purposeful and improves conception rates, which is also expressed in larger litters obtained, has been confirmed by Lorek et al. [1994] – the largest litters at birth were characteristic for dams which had been mated twice (single mating in the first period and the another one in the second period) and three times (single mating in the first period and two matings in the subsequent period of sexual activity). What is interesting, the authors did not see any significant differences between average litters of mink females mated according to various schemes, hence they recommend the double mating as the best choice. Moreover, the authors claim that triple mating of a dam in the same period does not increase the average litter size and does not allow reducing the population of male breeders, which in turn increases the production costs. Elofson et al. [1989] and Seremak et al. [2013] also demonstrated that double mating is more productive than any other system. The latter author reports that females attained better reproductive performance if mated twice, irrespective of the fur color variant.

On the other hand, Ślaska et al. [2009] confirmed that a higher number of matings positively correlated with the litter size at birth; the authors tried this conclusion on Scanblack and Pearl Cross mink. The authors claimed

that the highest levels of fertility, 7.94 and 7.84 born and live-born kits, respectively, were attained by mink dams mated five time during a single breeding season. Ślaska and Rozempolska-Rucińska [2011] report that not only does the litter size depend on the date and number of matings, but also on the intervals between subsequent matings; the best results were obtained if the period between the first and the last mating was possibly the shortest.

Our study also focused on the litter size at birth in relation to the mating date. The great differences in the number of offspring sired by males of different color variants may result from the fact that survival rates of the embryos depends on the time spent on the diapause – the sooner the conception, the longer the lag until implantation, or diapause, which affects the survival rate of the embryos. This hypotheses has been proposed by Venge [1973] and Yamaguchi et al. [2004]. As a consequence, the later it comes to mating with the subsequent male, the larger the litter it probably sires [Venge 1973]. Other authors [Sulik et al. 2007] also state that an effect of superfetation, present if we mate a female to a number of males, indirectly confirm that the date and number of matings is of importance in terms of litter size.

Also, if the mating (single or double) occurred in the first period only, or in the second period only, the difference in the average litter size was not that great. This may imply that a large percentage of embryos from the first-period mating die when the female is mated in the second period. Placental scar counting (PSC) is a good method of finding the actual number of embryos that had been implanted in the mink uterus [Elmeros and Hammershøj 2006]. This method, however, is not fully reliable, as it shows the scars of all the embryos implanted in the uterus.

ine wall (both those actually born and those that died after implantation), therefore diapause embryonic mortality may only be roughly estimated.

## CONCLUSION

The analysis of various mating schemes revealed that if we apply mating on two dates, most of the embryos conceived as a result of the first mating die. It can be concluded that in all the cases when females were mated on both the first and the second date, regardless of the number of matings, much larger litters were born after the matings on the second date.

## ACKNOWLEDGEMENTS

This work was financed from the statutory activity funds.

## REFERENCES

- Elmeros, M., Hammershøj, M. (2006). Experimental evaluation of the reliability of placental scar counts in American mink (*Mustela vison*). *Eur. J. Wildl. Res.*, 52(2), 132–135. DOI: 10.1007/s10344-005-0014-2.
- Elofson, L., Lagerkvist, G., Gustafsson, H., Einarsson, S. (1989). Mating systems and reproduction in mink. *Acta Agr. Scand.*, 39, 23–41. DOI: 10.1080/0001512890943-8496.
- Felska-Błaszczczyk, L. (2012). Effect of light day during pregnancy on the results of breeding American mink (*Mustela vison*, Schreb.) [Wpływ wydłużenia dnia świetlnego w okresie ciąży norki amerykańskiej (*Mustela vison*, Schreb.) na wyniki rozrodu]. *Wyd. Ucz. ZUT, Rozprawa habilitacyjna* [in Polish].
- Felska-Błaszczczyk, L., Seremak, B., Lasota, B., Sobczyk, J. (2012). Influence of gestation length and multiplicity of mating encounters in different color varieties of the American mink (*Mustela vison*) on selected parameters of reproductive performance. *Acta Sci. Pol. Zootechnica*, 11(3), 21–30.
- García, P. (2010). Female multiple copulation in the invasive American Mink (*Neovison vison*). *North-Western J. Zool.*, 6(1), 138–139.
- Lorek, M.O., Gugolek, A., Gawarecka, B. (1994). Observation of the heredity of fur colour in coloured minks [Observacje nad odziedziczalnością umaszczenia norek barwnych]. *Zesz. Nauk. Prz. Hod.*, 15, 217–218 [in Polish].
- Macdonald, D., Harrington, L.A. (2003). The American mink: the triumph of adaptation out of context. *New Zealand J. Zool.*, 30, 421–441. DOI: 10.1080/03014223.2003.9518350.
- Macdonald, D., Harrington, L.A., Yamaguchi N., Thom M.D.F. (2015). Biology, ecology and reproduction of American mink *Neovison vison* on lowland farm land. In book: *Wildlife Conservation on Farmland Volume 2: Conflict in the countryside* Chapter: 6 Publisher: Oxford University Press, Editors: Macdonald, Feber, 126–147.
- Roelling, K., Menzies, B.R., Hildebrandt, T.B., Goeritz, F. (2011). The concept of superfetation: a critical review on a “myth” in mammalian reproduction. *Biol. Rev.* 86, 77–95. DOI: 10.1111/j.1469-185X.2010.00135.x.
- Seremak, B., Siekierska, A., Felska-Błaszczczyk, L., Dziadosz-Styś, M., Lasota, B., Dworecka-Borczyk, M. (2013). Analysis of copulations in American mink females mated every day during the breeding season. *Acta Sci. Pol. Zootechnica*, 12(2), 49–56.
- Sulik, M., Seremak, B., Matyja, A. (2007). Analize of effect selected indices on litter size of minks in relation to colour variety. *Arch. Tierz., Dummerstorf*, 50(2), 214–219.
- Thom, M.D., Macdonald, D.W., Mason, G.J., Pedersen, V., Johnson, P.J. (2004). Female American mink, *Mustela vison*, mate multiply in a free-choice environment. *Anim. Behav.*, 67, 975–984. DOI: 10.1016/j.anbehav.2003.09.008.
- Venge, O. (1973). Reproduction in the mink. *Yearbook 1973. The Royal Veterinary and Agricultural University Copenhagen*, 95–146.
- Yamaguchi, N., Sarno, R.J. Johnson, W.E., O’Brien, S.J., Macdonald, D.W. (2004). Multiple paternity and reproductive tactics of free-ranging American minks, *Mustela vison*. *J. Mammal.*, 85(3), 432–439. DOI: 10.1644/1383939.
- Ślaska, B., Rozempolska-Rucińska, I. (2011). Mating system and level of reproductive performance in mink (*Neovison vison*). *Ann. Anim. Sci.*, 11(1), 105–113.
- Ślaska, B., Rozempolska-Rucińska, I., Jeżewska-Witkowska, G. (2009). Variation in some reproductive traits of mink (*Neovison vison*) according to their coat colour. *Ann. Anim. Sci.*, 9(3), 287–297.

## SYSTEM KRYCIA A LICZBA MŁODYCH U NOREK HODOWLANÝCH (*NEOVISON VISON*) – ZJAWISKO WIELOOJCOSTWA

### STRESZCZENIE

Celem pracy była analiza różnych systemów i terminów kryć oraz liczby kojarzeń ze względu na liczebność miotu. Materiał do badań stanowiły: jednoroczne samice norki amerykańskiej dwóch odmian barwnych: perła (P) i biała Hedlunda (WH), jednoroczne samce norki amerykańskiej 4 odmian barwnych: perła (P), biała regal (WR), biała Hedlunda (WH) i czarny krzyżak (BC). Odmiana barwna samców została tak dobrana, aby w łatwy sposób można było określić, po jakich samcach i ile potomstwa urodziło się z poszczególnych kryć w danym terminie. Zwierzęta w stadzie podstawowym kojarzone były według różnych schematów krycia. W wyniku analizy stwierdzono, że przy kryciu w dwóch terminach większość zarodków pochodzących z krycia w pierwszym terminie zamiera.

**Słowa kluczowe:** norka amerykańska, rozród, system kojarzeń