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Effect of photoperiod modulation in American mink males on their testosterone concentrations and mating performance

Wpływ modulacji świetlnej na koncentrację testosteronu we krwi i wyniki krycia u samców norki amerykańskiej

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Abstract

The aim of the study was observation of the effect of light modulation on blood testosterone levels and libido in male American mink. The study was performed on 64 Black Velvet male mink on a farm located in the north-west of Poland. Day length was modified using artificial lighting placed above mink cages. Two variants of lighting programs were applied to males: (I) from 8 of February and (II) from 12 of February. In order to measure blood testosterone concentration samples were collected from males of the experimental groups and the control groups (animals kept under natural light conditions). As a result of the experiment, the males subjected to extended lighting were found to have had slightly higher libido than those outside the treatment, although no strong or clear effect of lighting on the pattern of testosterone levels in male mink has been found. It remains unexplained whether slightly, non-significantly higher libido of the experimental males is related to the applied modulation of photoperiod. In order to unambiguously clarify the effect of day light modulation on the results of males' sexual activity, further studies should be performed on a larger population and possibly using modified extended lighting programs.

Keywords: American mink, light modulation, reproduction, testosterone

Streszczenie

Celem badań była określenie wpływu modulacji świetlnej na koncentrację testosteronu we krwi i libido samców norki amerykańskiej. Badania wykonano na 64 samcach odmiany Black Velvet utrzymywanych na fermie w północno-zachodniej Polsce. Długość dnia modyfikowano, stosując sztuczne oświetlenie umieszczone nad klatkami. Zastosowano dwa programy świetlne dla samców: (I) od 8 lutego i (II) od 12 lutego 12. W celu oznaczenia koncentracji testosteronu we krwi pobierano jej próbki od samców w grupie doświadczalnej i kontrolnej (norki utrzymywane w naturalnych warunkach świetlnych). Samce przebywające w zmodyfikowanych warunkach świetlnych wykazywały nieco większe libido niż samce z grup kontrolnych, jednak nie stwierdzono silnego i jednoznacznego wpływu doświetlania na koncentrację testosteronu we krwi samców. Niewyjaśnionym pozostaje, czy nieznacznie większe, statystycznie nieistotne libido samców doświadczalnych jest związane z zastosowaną modulacją świetlną. Jednoznaczne wyjaśnienie wpływu modulacji świetlnej na aktywność płciową samców wymaga dalszych badań na większej populacji zwierząt i ewentualnie z zastosowaniem innych programów doświetlania.

Słowa kluczowe: modulacja świetlna, norka amerykańska, reprodukcja, testosteron

Introduction

Several biotechnical methods are in use by breeders to achieve optimum mating organization. In mink females these include e.g. the control of mating behaviors by manipulating light (Felska-Blaszczyk et al., 2013). There is little information in the literature of the subject on the light regulation of reproductive processes in male mink. It is obvious that day length in seasonally breeding animals is a key factor in their reproduction processes, which is reflected also in the gonadal hormonal activity.

Melatonin, synthesized in the pineal gland, is a biochemical signal that informs the body about a changing day length. Melatonin is involved in the transition of the light signal into neuroendocrine information. In short-day animals, elongation of the night intensifies melatonin secretion, which stimulates the secretion of gonadotropins by the pituitary gland. Increased melatonin secretion is the signal to start sexual activity (Jaśkowski and Zdunczyk, 2007; Zieba et al., 2011).

According to Sotowska-Brochocka (2001) light programs may be used to induce changes in the physiological processes running in the natural annual reproduction cycle. The author stated that photoperiod manipulations, ie. its elongation or shortening, may in the species breeding seasonally lead to physiological changes that would mimic the seasonality occurring in the natural annual cycle. For example, photoperiod changes in adult common hamsters lead to variations in the LH level (Bronson, 1988).

According to Bilińska (2002), a well-designed light program can delay or even inhibit the gonadal differentiation.

Boissin-Agasse et al. (1982) applied several photoperiod programs and observed the blood plasma concentration of testosterone and testicular volume of male mink. They maintained an artificial photoperiod for 75 days, from October, or for 150 days, from

November, and noticed that minimum 16 hours of darkness is needed to stimulate the gonads. As soon as the dark phase was interrupted, the testicular development stopped. The highest serum testosterone concentration was observed in mink housed under unchanging light conditions, i.e. at 4 hours of light per day. The studies by Forsberg et al. (1989) on silver fox and Gulevich et al. (1995) on mink confirmed these findings and demonstrated that artificial lighting results in changes in testicle size and blood testosterone concentrations. According to unpublished data (personal communication), in Canada a program for males is applied consisting in gradual extension of light phase by an hour starting three weeks prior to breeding season; this has been practiced for many years. Available sources (Pelletier, 1986) allow concluding that the extension of day light phase in February is a common practice, although no published scientific report has been found that would specify the details and outcomes of such a light modulation program.

This study, which focused on extending the day prior to the mating season, was to answer the question, whether additional light applied to males before breeding will positively affect the reproduction performance in the geographic location of Poland, as is the case in Canada. If so, this could be used as a base to design an optimal lighting program. The direct aim of this study was to determine the effect of photoperiod modulation on blood testosterone levels and libido in male American mink.

Materials and methods

Animals

The study was carried out on 64 males of farm mink (Black Velvet color variant), aged 11 months, similar in body weight and length, managed and fed under the same conditions and housed on a mink farm located in the north-western part of Poland (53° 46′ 03″ N; 15° 09′ 23″ E). The animals were fed according to current recommendations (Gugolek and Barabasz, 2011). Semi-liquid feed based on fish and chicken was supplied three times a day, automatically dosed by a machine feeder directly on top of the cage. Each animal received the same ration of feed of the same quality.

Both treatment and control animals remained in so called breeding sets. Light-treated males were hosed in the same shed, whereas control males remained in another one, where no additional lighting was applied.

The males were brothers or half-brothers and the females were sisters or half-sisters. The animals were assigned to the following groups:

- C1 and C2 (control, natural light conditions), 16 males each,
- E1 (treated with light program I), 16 males,
- E2 (treated with light program II), 16 males.

Photoperiod modulation

In order to control the light in the shades of the experimental animals, artificial light (700 lm) was installed above the cages. The natural length of day at the location of the farm on 8 February is 9 hours and 20 minutes, and increments daily by 4 or 5 minutes due to an earlier sunrise and a later sunset. On 28 February, the natural day length is 10 hours and 45 minutes. The sunrise was defined as the moment when the upper edge of the solar disc crosses the geocentric (real) horizon, whereas the sunset was assumed as the moment when the upper edge of the solar disc disappears behind the horizon. Two programs of modified day length were applied (Figure 1):

- Program I: the first day (8 Feb.) was extended by 52 minutes. The subsequent days were extended gradually, so that on the last day of light modification (28 Feb.) the day was 11 hours and 55 min long, i.e. by 1 hour and 10 min longer than the natural day. The number of days artificially modified was 21. The intercept between the end of lighting and beginning of matings was 1 day.
- Program II: the first day (12 Feb.) was extended by 55 minutes. The subsequent days were extended gradually, so that on the last day of light modification (28 Feb.) the day was 11 hours and 54 min long, i.e. by 1 hour and 9 min longer than the natural day. The number of days artificially modified was 17. The intercept between the end of lighting and beginning of matings was 4 days.

On the last day of both light programs, I and II, the artificially extended light phase corresponded to the natural length of day light on 16 March.

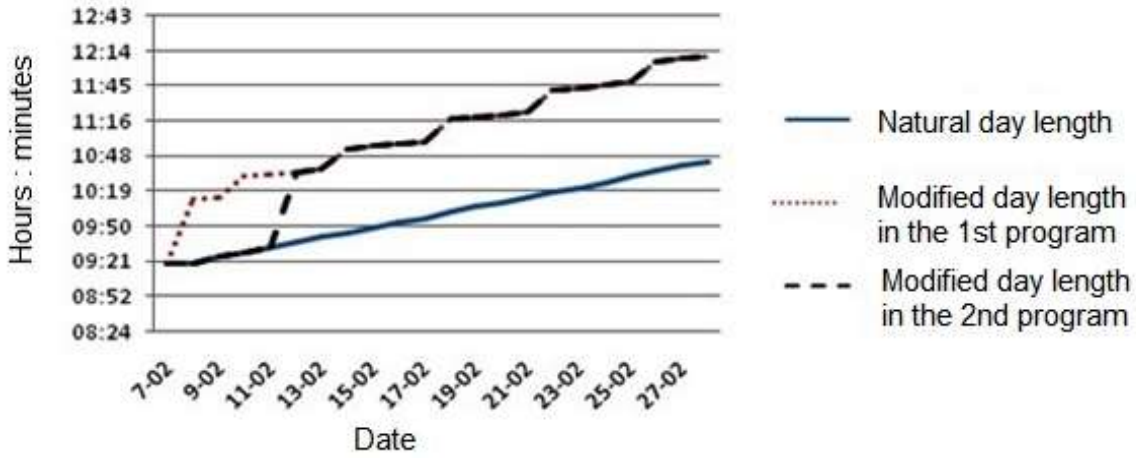


Figure 1. Comparison of the natural and modified day lengths in the first and second lighting program

Blood sampling and plasma testosterone testing

Blood samples for testing testosterone concentrations were collected from all the experimental animals before the commencement of the lighting program (Table 1). Testosterone levels were measured again after 10 days (program I) or after 6 days

(program II) from the introduction of light extension program. Sampling dates are given in Table 1. Blood samples were collected according to the procedure described in a previous paper (Lasota et al., 2013).

Plasma testosterone concentration was measured by the immunofluorescent method, using the DELFIA kits (PerkinElmer, Wallac Oy, Finland). Testosterone readings were carried out using the Victor reader (Wallac Oy).

Table 1. Blood collection scheme in both light programs

	Program I		Program II	
	Before lighting			
	On 4 February		On 2 February	
Number of males	32		32	
	During the lighting (18 February)			
	Treatment (E1)	Control (C1)	Treatment (E2)	Control (C2)
Number of males	16	16	16	16

Evaluation of male libido

Observations of mating always involved the same suitably trained staff. Males mated females of the same group. The female was admitted to a male for approx. 1 hour. The effective mating was noted if animals clutched the males holding the female by the top of her head. Copulations occurred twice daily (morning and evening). Both males and females had no prior breeding experience. Animals showing aggression were excluded.

The libido of males was determined as the number of mating occurrences over the first five days of the mating season.

The number of males from each group that began copulating on the subsequent days of the season were also assembled. The results of mating were analysed in 16 males from either treatment group and in 16 males from either control group. Males began to mate from 2nd March in program I, and from 5th March in program II.

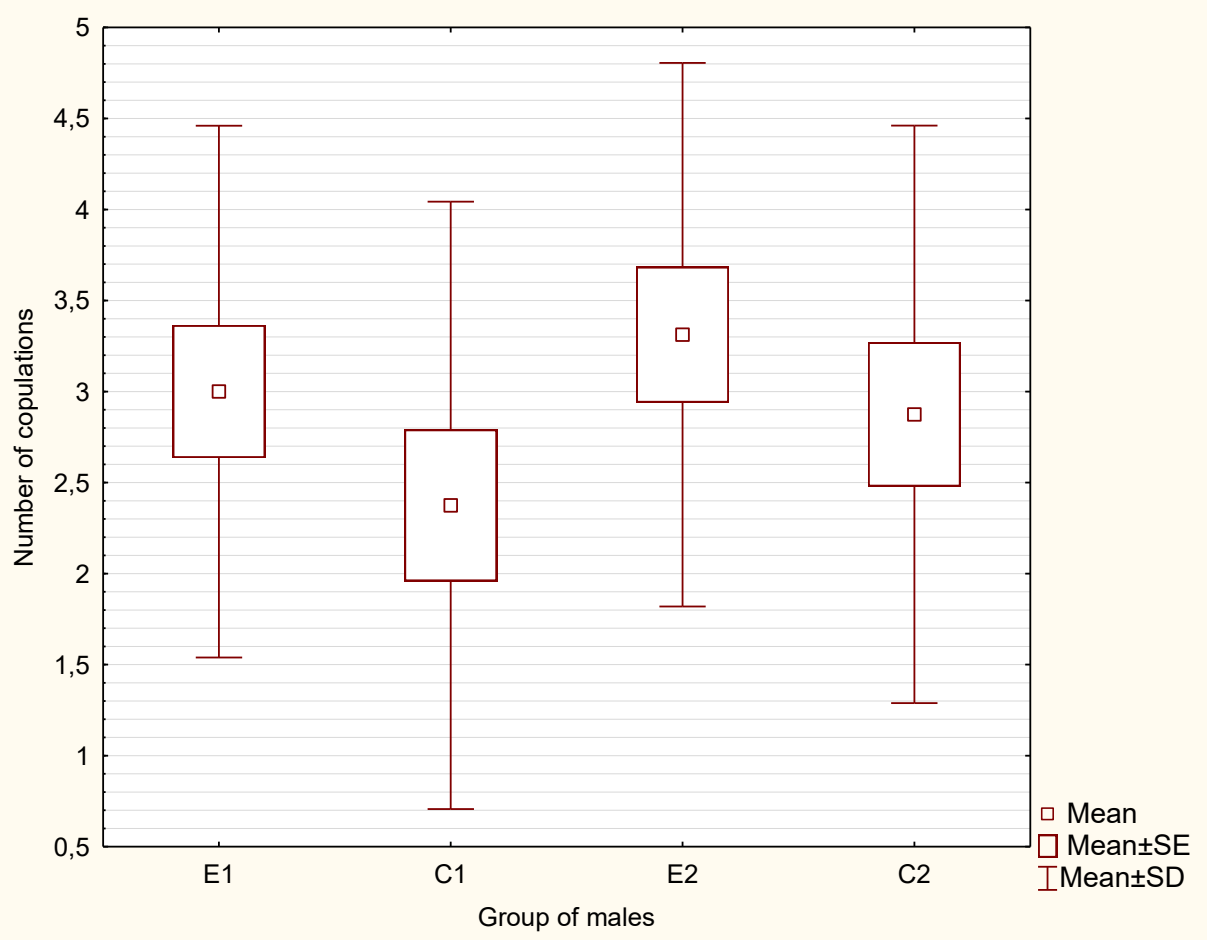
Statistical analysis

The results were subjected to statistical procedures using one-way ANOVA. The significance of the differences was tested with chi-square and Tukey's tests. A correlation matrix was also calculated. The analyses were carried out using the STATISTICA 9.0 PL software package.

Results

Mating results

As can be concluded from the graph in Figure 2, the treatment males served a higher number of females on average compared with the control, both under lighting program I and II (an average of 3 and 3.31 mating occurrences, respectively). The mean number of services by control males was 2.37, in program I, and 2.87, in program II. The differences, however, were non-significant.

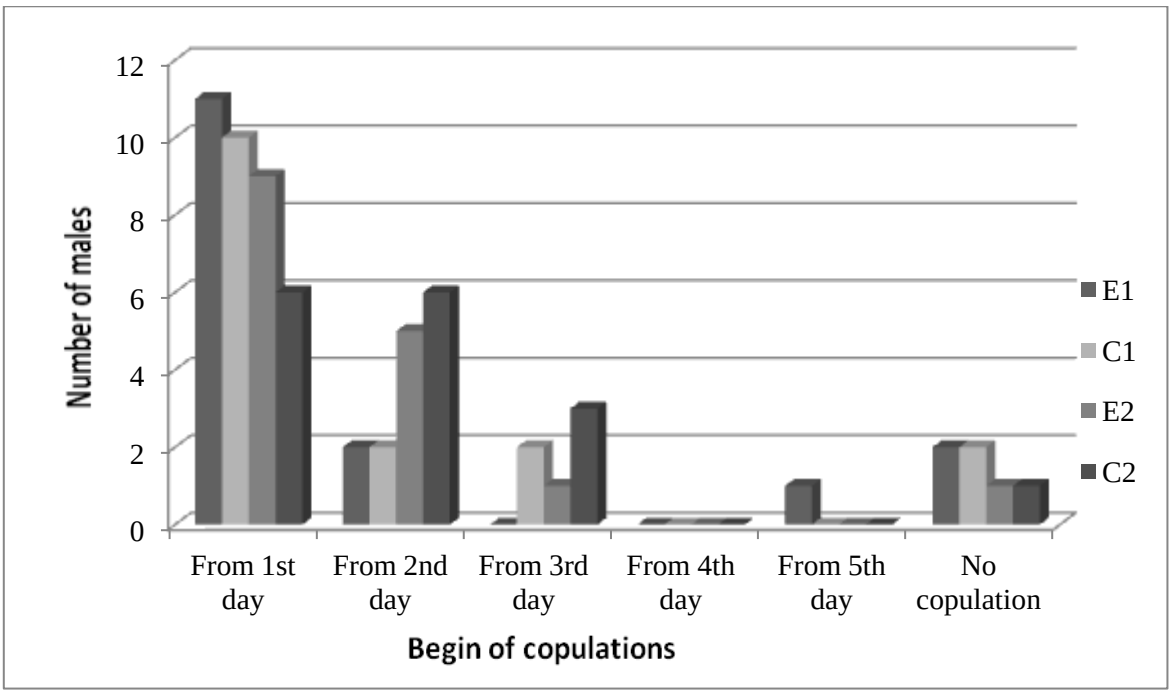


E1, E2 – experimental group of males in lighting program I or II; C1, C2 – control group of males in lighting program I or II.

Figure 2. Average number of copulations by males during the five days of mating period by group

In both programs, most males mated from the first day of the season, both in the treatment and control groups. Figure 3 presents the number of males from each group that began mating on the subsequent days of the experiment. From the very first day, 11 treatment males copulated in program I and 9 in program II. Control males mated slightly worse from the first day, 10 in program I and 7 in program II. In the program I, both in the treatment and the control groups, 2 males each failed to

copulate, whereas in the program II – one male each group. None of the observed differences proved to be statistically significant.

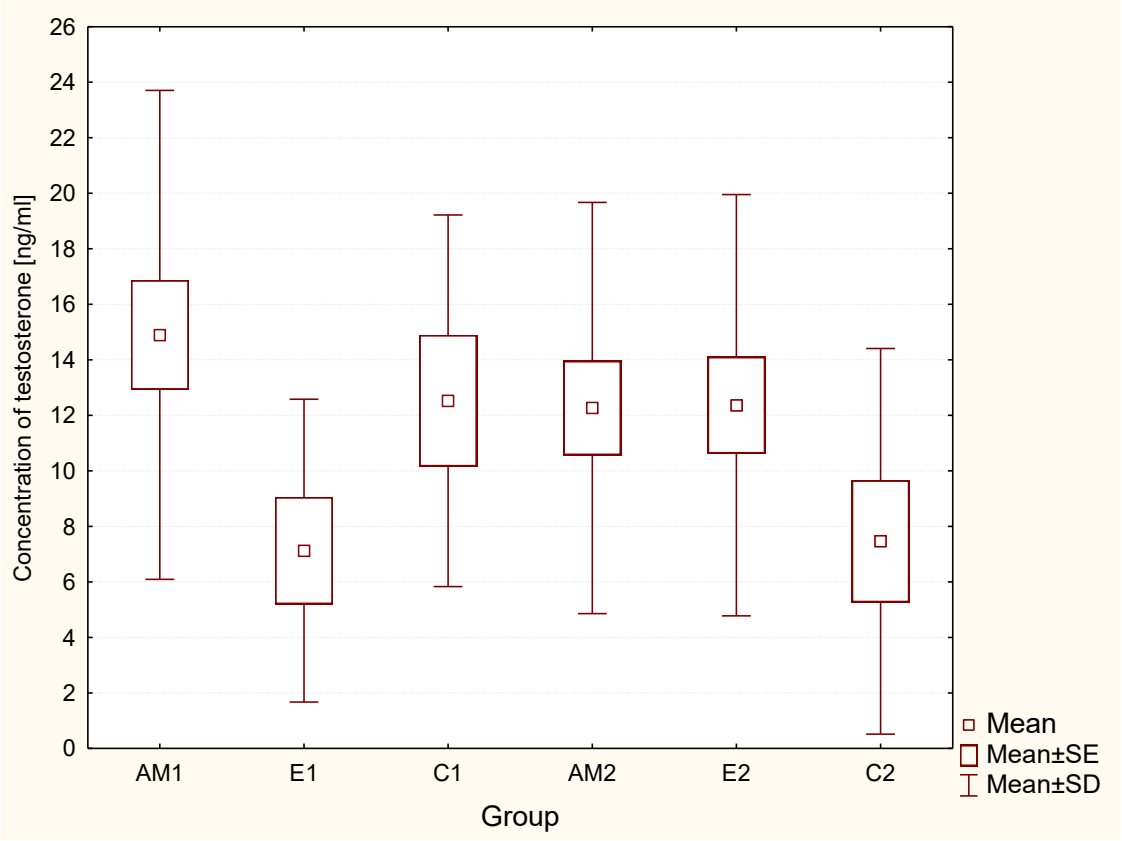


E1, E2 – experimental group of males in lighting program I or II; C1, C2 – control group of males in lighting program I or II.

Figure 3. Number of males that started copulations on the subsequent day of the mating period

Testosterone levels under modulated day light

In the first program, the average testosterone concentration during day extension in the control group males was higher than in experimental animals and slightly lower than before the photoperiod modulation had been applied (Figure 4). In the second lighting program the situation was reversed; males from the experimental group had a similar average concentration of testosterone on 18th February as at the beginning of February, before the lighting period. In contrast, the average blood concentration of testosterone in the control group of males in the second half of February was lower. No significant differences were found between the results of the six mentioned groups.



AM1, AM2 – group of males before introducing the modulation of lighting in lighting program I or II; E1, E2 – experimental group of males during the modulation in lighting program I or II; C1, C2 – control group of males (no extended lighting) in lighting program I or II.

Figure 4. Average blood concentration of testosterone in males before starting and during light modulation in both programs

Discussion

Photoperiod modulation

Studies on the possible control of reproduction processes in males through modified day length were carried out on rams by Lincoln and Davidson (1977). The authors applied alternating periods: a “long” day (16 hours of light : 8 hours of dark), which lasted 16 weeks, followed by a “short” day (8 hours of light : 16 hours of dark), which again lasted for another 16 weeks and was again followed by the “long” day period. The authors observed how the gonads and blood concentrations of hormones changed under the influence of the alternating light programs. The rams were sexually inactive during the “long” day regime. Following a rapid shortening of the day, however, blood concentration of gonadotropins increased within a couple of weeks. Another “long-day” period resulted in gonadal involution and reduced testosterone concentration in the blood (Lincoln and Davidson, 1977). Also, studies carried out on American mink by Boissin-Agasse et al. (1982). Such relationships have not been observed in the presented studies and were reversed in each lighting program. Artificial day shortening or extending has an enormous effect on male reproductive abilities in polar foxes; males subjected to a short, 6-hour day from

February through June had twice as high testosterone level as males remaining under the natural photoperiod (Forsberg et al., 1989).

The aim of the applied photoperiod modulation was to accelerate the readiness of males to mate, so that they would be interested in females from the first days of the admittance. Mating season on a mink farm lasts up to three weeks, so very shortly if compared with other fur animals. Ideally, a female should copulate four times; however, due to many reasons, some females mate only ones, and some twice or three times. This may be due to a late first mating, as late as during the last days of the season. From an economic point of view, breeders care that all females be mated as soon as possible, since males may be skinned still having winter coat on. In the experiment carried out in this work the males kept under modulated photoperiod were characterized by slightly and non-significantly stronger libido. Considering experimental males, this result could possibly be explained with the span between the completion of lighting and the first day of mating (1 day in program I and 4 days in program II). However, comparing the libido of control males, in which the observed relationships were similar to males of the experimental groups, brings a suggestion that the differences in mating outcomes between the photoperiod variants may have resulted from other factors that, perhaps, conceal the effect of light. Nutrition factors should be excluded, as these were equal within the entire farm. The remaining factors, on the other hand, have not been included in the study. The most likely explanation, which does not exclude others, seems the commencement of the mating.

Effect of light modulation on testosterone concentrations

Previous research by authors of this work (unpublished), as well as studies by Sundqvist et al. (1984), reveal that testosterone concentration drops in February and March. Onderka (1996) stated that a sharp reduction in testosterone concentration intensifies the sexual drive. In this view, it can be assumed that the average testosterone level in experimental males should be lower than in control ones due to the slightly higher libido. The results of testosterone measurements, however, do not support this assumption. Lack of a strong and clear effects of lighting on the pattern of testosterone levels in male mink observed in this study may confirm the opinion of Messager et al. (1997), who conclude that in the period from November to February, when the hypothalamic-pituitary-gonadal axis is already functional, a decline in serum testosterone is not dependent on photoperiod, due to the effect of photoresistance observable during this time. Assuming that the observations by these authors are accurate, extension of the day in February had no effect on the changes in the concentration of testosterone.

Conclusions

It remains unexplained whether slightly, non-significantly higher libido of the experimental males is related to the applied modulation of photoperiod.

No strong and clear effect of extended lighting on testosterone levels in mink males has been found.

In order to unambiguously clarify the effect of day light modulation on the results of males' sexual activity, further studies should be performed on a larger population and possibly using modified extended lighting programs.

References

Bilińska, B. (2002) New insights into the cellular site of androgen aromatization and the role of estrogens in the rodent testis. Progress in Andrology, 4, 27-43.

Boissin-Agasse, L., Boissin, J., Ortavant, R. (1982) Circadian photosensitive phase and photoperiodic control of testis activity in the mink (*Mustela vison* Peale and Beauvois), a short-day mammal. Biology of Reproduction, 26, 110-119. DOI: https://doi.org/10.1095/biolreprod26.1.110

Bronson, F.H. (1988) Mammalian reproductive strategies: genes, photoperiod and latitude. Reproduction Nutrition Development, 28, 335−347. DOI: https://doi.org/10.1051/rnd:19880301

Felska-Błaszczyk, L., Seremak, B., Lasota, B., Klecha, A. (2013) Extra light during pregnancy improves reproductive performance of mink (*Neovison vison*). Annals of Animal Science, 13, 797–805. DOI: https://doi.org/10.2478/aoas-2013-0054

Forsberg, M., Fougner, J.A., Hofmo, P.O., Madej, M., Einarsson, E.J. (1989) Photoperiodic regulation of reproduction in the male silver fox (Vulpesvulpes). Journal of Reproduction and Fertility, 87, 115-123. DOI: https://doi.org/10.1530/jrf.0.0870115

Gugołek, A., Barabasz, B. (2011) Zalecenia żywieniowe i wartość pokarmowa pasz Zwierzęta futerkowe. Jabłonna: Instytut Fizjologii i Żywienia Zwierząt im. Jana Kielanowskiego PAN.

Gulevich, R.G., Klotchkov, D.V., Ivanova, L.N., Osadchuk, L.V. (1995) Gonadal function in mink under artificial photoperiods. Journal of Reproduction and Fertility, 103, 147-152. DOI: https://doi.org/10.1530/jrf.0.1030147

Jaśkowski, J., Zduńczyk, S. (2007) Biologiczne uwarunkowania wartości rozrodowej samca. In: Strzeżek, J., ed. (2007) Biologia rozrodu zwierząt. Olsztyn: Wydawnictwo UMW, 133-142.

Lasota, B., Masłowska, A., Felska-Błaszczyk, L., Dziadosz, M., Seremak, B., Skuratko, A. (2013) Stimulatory effect of hCG on male American mink (*Neovison vison*) in the breeding season. Annals of Animal Science, 13, 563–570. DOI: https://doi.org/10.2478/aoas-2013-0026

Lincoln, G.A., Davidson, W. (1977) The relationship between sexual and aggressive behavior, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. Journal of Reproduction and Fertility, 49, 267- 276. DOI: https://doi.org/10.1530/jrf.0.0490267

Messager, S., Caillol, M., George, D., Martinet, L. (1997) Seasonal variation of melatonin binding sites in the pars tuberalis of the male mink (*Mustela vison*). Journal of Neuroendocrinology, 9, 523-528. DOI: https://doi.org/10.1046/j.1365-2826.1997.d01-1122.x

Onderka, D. (1996) Male reproductive system. In: Hunter, D.B., Lemieux, N., eds. (1996) Mink… biology, health and disease. Guelph: Graphic and Print Services, University of Guelph.

Pelletier, R.M. (1986) Cyclic formation and decay of the blood-testis barrier in the mink (*Mustela vison*), a seasonal breeder. American Journal of Anatomy, 175, 91-117. DOI: https://doi.org/10.1002/aja.1001750109

Sotowska-Brochocka, J. (2001) Fizjologia zwierząt. Wybrane problemy. Warszawa: Wydawnictwa Uniwersytetu Warszawskiego.

Sundqvist, C., Lukola, A., Valtonen, M. (1984) Relationship between serum testosterone concentrations and fertility in male mink (*Mustela vison*). Journal of Reproduction and Fertility, 70, 409-412. DOI: https://doi.org/10.1530/jrf.0.0700409

Zieba, D.A., Kirsz, K., Molik, E., Romanowicz, K., Wojtowicz, A.K. (2011) Effects of orexigenic peptides and leptin on melatonin secretion during different photoperiods in seasonal breeding ewes: An in vitro study. Domestic Animal Endocrinology, 40, 139–146. DOI: https://doi.org/10.1016/j.domaniend.2010.09.008