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STIMULATORY EFFECT OF HCG ON MALE AMERICAN MINK (*NEOVISON VISON*) IN THE BREEDING SEASON*

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Abstract

The aim of the study was to analyse the effect of a male mink single-dose hCG stimulation on the libido during the mating season and on blood testosterone levels after the season. The material involved males of American mink. The treatment-group males were administered a dose of 100, 150 or 200 U of hCG. Blood was collected twice, approx. 2 weeks prior to and on the completion of the mating season. The group receiving 100 U hCG had the highest percentage of males effectively copulating with females within the first 24 hours after stimulation, whereas males stimulated with a dose of 200 U hCG showed the lowest libido over the same period. On the other hand, males of the group stimulated with 150 U hCG mated to the highest number of females throughout the mating season. The mean plasma testosterone concentration in all the studied males on 18 February was 12.44 ng/ml. The drop in testosterone concentration at the end of the mating season was significant.

Key words: mink, *Neovison vison*, fertility regulation, mating effect, reproduction

Hormonal female reproduction control in some livestock animals (e.g. cattle or swine) has long been applied as a standard procedure within the breeding technology. Attempts have also been made, with varying success, to apply hormones to regulate the breeding of goats (Restall et al., 1995; Baran et al., 2003), a species showing seasonality in reproduction, as well as in mares (Lofstedt, 1986). Also in female mink, human chorionic gonadotropin (hCG) was used in order to enhance their fertility and prolificacy (Rietveld, 1978; Klotchkov and Eryuchenkov, 2003).

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Administration of hCG induces rapid stimulation of testicular endocrine function in males of a number of animals, as demonstrated in bulls (Sundby and Torjesen, 1978), rats (Hodgson and Kretser, 1982), rabbits (Rebollar et al., 1998), marmosets (Kholkute et al., 1983), boars (Bonneau et al., 1982; Oskam et al., 2010), and rams (Garnier and Saez, 1980). According to these reports, a single injection of hCG induced a marked increase in blood level of testosterone. A study by Matsumoto et al. (1983), carried out on human males, was aimed to determine the effect of long-term (several months') administration of high doses (such as 5000 U) of hCG on the male reproductive system. The study revealed that the hormone significantly increased sperm production as estimated based on sperm concentration and their morphology. It was concluded that hCG could be successfully used to induce and sustain the spermatogenesis; this had already been shown by Johnsen (1978), who stated that hCG was able to sustain the process of spermatogenesis for a long time.

In addition to hormonal stimulation of individuals with impaired reproductive function, research has also involved healthy subjects, in order to determine the effect of hCG on the functioning of the body, especially the reproductive system. Sundby and Torjesen (1978) studied the effect of an injection of the hormone on plasma testosterone levels in four bulls. They found that after administration of a single dose of 6000 U hCG, testosterone levels rose and remained at elevated levels from 9 to 13 days. Similar studies were carried out in male mice subjected to hCG stimulation. Mice were subcutaneously administered a dose of 10 U of hormone, which resulted in an increase of concentrations of testosterone and 5α-dihydrotestosterone in the bloodstream. In response to hCG stimulation, rapid androgens synthesis occurred which continued for over 96 hours (Lau et al., 1978). By contrast, Garnier and Saez (1980) studied the effect of hCG stimulation of the testes of rams. After a dose of 6000 U hCG administered to four rams, within 2 hours there was a sharp increase in testosterone levels, from 0.81 to 3.76 ng/ml. After 10 hours, a decrease to the level of control was observed, despite the still high level of hCG. In an experiment on rats, which received hCG at a dose of 100 U, a significant increase in both testosterone concentration and testicular blood flow was observed (Damber et al., 1981).

According to all the cited reports, an increased level of testosterone in response to hCG stimulation was observed, which lasted depending on the species from several hours to several days. Testosterone also positively affects the libido and erection. Reyes-Vallejo et al. (2007) demonstrated that application of testosterone resulted in 45% to 73% increased erection functions in men, depending on its initial concentration. The authors also observed from 29.8% to 96.6% increase in libido, in relation to its initial level.

Sundqvist et al. (1984) observed a correlation between testosterone concentration and fertility in mink. The literature, however, lacks information on male mink in terms of hCG stimulation.

The aim of the study was i) to analyse the effect of a single-dose hCG stimulation of male mink on their libido, as measured with the number of mating acts observed on the following day and within 2 weeks after the stimulation, and ii) to investigate whether the single hCG stimulation affects the concentration of testosterone in blood after the completion of the mating season.

Material and methods

Animals and hormonal stimulation

The material comprised males of Sapphire American mink (*Neovison vison*) in the first year of their farming, which did not show any clinical symptoms of a disease. The animals were housed under the same conditions on a large production farm. The males were divided into two groups: treatment (30 males) and control (24 males). On 4 March, the males in the treatment group were intramuscularly administered a dose of human chorionic gonadotropin (hCG) (Chorulon, Intervet, Poland): 14 males received a dose of 100 U, and 8 males each got 150 or 200 U of the hormone. After 24 hours from the administration, the males of both treatment and control groups were allowed to females. The males were mated to females of the same colour (Sapphire). The effect of the stimulation was measured as the number of females effectively mated on the following day and within the 2-week period following the stimulation. All males involved in the study were admitted to females every day. The females were considered effectively mated if they finally gave birth to offspring.

In order to study the effect of a single stimulation with hCG on the level of testosterone at the end of the mating season, blood was collected twice from 8 males from each treatment group and the control group: approx. 2 weeks prior to mating (18 February), and on the completion of the mating period (18 March).

Blood collection

Blood from males was collected from toenails of the rear legs, according to the procedure applied for Aleutian disease testing (Nova Scotia Agriculture). The collections were carried out before feeding, between 9:00 and 11:30. Blood samples were centrifuged within 1.5 to 3 hours after collection, and the resulting plasma was stored at –20°C until analysed.

The blood sampling procedure was duly permitted by the Local Ethical Committee for Experiments on Animals.

Testosterone determination

Plasma concentration of testosterone was measured by immunofluorescent method, which is based on the effect of element fluorescence, using the DELFIA kits (Perkin-Elmer, Wallac Oy, Finland). The DELFIA fluoroimmunoassays are based on the competition between Eu-labelled testosterone and non-labelled sample testosterone for the binding sites on the antibody molecule. The number of Eu-labelled hormone antibodies is constant, whereas the concentration of the non-labelled hormone is the function of creating the antibody-labelled hormone complex. Based on this, a standard curve has been drawn for reading concentration of the hormone in a given sample.

Statistical analysis

Testosterone concentration in blood and the number of effectively mated females were analysed by a one-way ANOVA. The significance of differences was tested using the Tukey test (RIR), for testosterone concentration, or chi-square test, for

the number of mating acts. Correlation tables allowed evaluating the relationships between testosterone concentrations before and after hCG stimulation. The statistical computations were carried out using Statistica 9.0 software package (StatSoft Polska).

Results

On the day that followed the administration of hCG to the experimental group individuals, all the males – including those in the control group – were allowed to females. As follows from Table 1, the group stimulated with 100 U of HCG was characterized by the largest percentage mating of males. On the other hand, the lowest libido was observed in the group of males stimulated with 200 U. The percentage of mating males in this group was even lower than in the unstimulated group. The chi-square test revealed no significant differences.

Table 1. The number of males mating within 24 hours after hCG stimulation

Stimulation variant	Total no. of males	No. of males mating within 24 hours after stimulation	Rate
100 IU	14	13	92.85%
150 IU	8	7	87.5%
200 IU	8	5	62.5%
Total hCG stimulated	30	25	83.33%
Non-stimulated	24	18	75.0%

Table 2. The number of females effectively mated by stimulated and non-stimulated males until 17 March

Stimulation variant	No. of males	No. of effectively mated females	Mean number of mated females per male
100 U	14	133	9.5±3.13
150 U	8	92	11.50±2.29
200 U	8	74	9.25±3.76
Total hCG stimulated	30	299	9.96±3.10
Non-stimulated	24	213	8.87±3.97

The number of females mated by hCG-stimulated males within 2 weeks was greater as compared with the control (Table 2). The comparison of the entire hCG-stimulated group males with the group of non-stimulated males revealed that a male of the former group mated effectively with slightly more than one female more on average.

The difference between the 200 U of hCG-stimulated males and non-stimulated males was nearly 10%. The highest difference was found between the group of 150 U of hCG-stimulated males and non-stimulated males – here the difference was

approx. 23% (2.62 mated females) in favour of the stimulated group males. All the observed differences, however, were statistically non-significant.

The mean plasma testosterone concentration in all the studied males (both in the treatment and the control groups) measured in blood collected on 18 February was 12.44 ng/ml. The differences within the treatment group were low, statistically non-significant (from 10.88 to 12.1 ng/ml), hence the treatment group was treated as a single comparison group in the statistical analysis. Testosterone levels in the control group were higher compared to the hCG-stimulated group, though the difference was also non-significant (Table 3).

Table 3. Plasma testosterone concentration in males before hCG stimulation and after the mating season

Group	n	Testosterone level before stimulation	Testosterone level after mating
hCG-stimulated	24	11.49 A±6.86	2.72 A±2.69
Control	8	13.39 b±10.93	7.06 b±8.07

Explanation: values marked with the same letters differ significantly at (A) $P<0.01$ or (b) $P<0.05$.

The relationships between the groups in testosterone concentrations in blood collected on 18 March remained similar to those on the completion of the mating season; the concentrations within the treatment group were virtually the same and ranged within 2.56 to 2.89 ng/ml. Therefore, like in the case of testosterone concentration measured on 18 February, the group was treated as one in the statistical analysis.

The highest testosterone concentration was observed in the control group, and the differences between the treatment groups and the control before stimulation were statistically non-significant (Table 3). The drop in blood plasma testosterone concentration in both groups was significant and reached from about 53% in the control group to approx. 76% in the group of stimulated males. It should be stressed in this place that the variability (variance) within the control group – both before and after stimulation – was high.

Statistical analysis revealed a significant correlation between testosterone levels before and after mating season ($r = 0.736$).

Discussion

Studies on stimulation of male American mink with human chorionic gonadotropin are rare. No reports on this subject were found in the literature, thus it is difficult to refer to the results of other authors. Generally, the present study shows a beneficial effect of stimulation of male mink with hCG, as measured by the criteria adopted in this work; however, the significance of the differences that occurred in any case have not been statistically confirmed. It seems that the dose of hCG is important for the stimulation. Since no guidance was found in the literature on the dose of hCG that could be applied for mink, we used doses based on analogy to other species. Chan-

drasekharam et al. (2003) used doses of 5 to 50 U in rats, which may be indicative of about 16 to 160 U/kg body weight. Rebollar et al. (1998) used a dose of 50 U of hCG in male rabbits, and Kholkute et al. (1983) in marmosets applied 40 or 80 U per animal, which – considering the average body weight of this species – results in about 100–200 U/kg. In the present study the doses of administered hCG were 100, 150 and 200 U, which gives an estimated approx. 33, 50 and 66 U/kg body weight.

Based on the efficiency of mating, both within 24 hours and within 14 days after stimulation, the results may suggest that the highest dose (200 U hCG) is not appropriate for mink. Stimulation with lower doses, 100 or 150 U, gave better results. However, in the experiment by Matsumoto et al. (1983), increased semen production in men was the result of high doses of hCG applied for several months. Sundby and Torjesen (1978) showed that increasing the concentration of testosterone is dependent on the dose of hCG. At a dose of 6000 U of hCG in bulls, the state of elevated testosterone lasted from 9 to 13 days. This suggests that the dose of hCG is the key factor for males and regulates the duration of its impact on male reproductive system. It cannot be excluded, however, that there is no universal dose of the hormone per unit of body weight for any species. The values quoted above tend to reflect the species specificity of the response to hCG. Nor can it be ruled out that the pattern of temporal changes in increased secretion of testosterone is different in males of various animal species. For example, rats revealed a biphasic pattern of testosterone secretion in response to a single dose of hCG (Hodgson and Kretser, 1982). Cited authors noted that there are two peaks of testosterone secretion, varying depending on the dose of the hormone. Also in rams (Garnier and Saez, 1980) and in marmosets (Kholkute et al. 1983), studies revealed wave-shape fluctuations in testosterone after injection of hCG. In order to clarify the optimal dose for male mink and to find a model of changes in testosterone after hCG stimulation, further research is needed.

In the second part of the study, we compared the concentration of testosterone before hCG stimulation and after the mating season. To avoid possible stress related to blood sampling, which could have disrupted the male libido, blood was not sampled during the mating season. As expected, on the completion of the mating period (18 March), a significant decrease in testosterone concentration was noted, but the extent of the decrease was different between the groups: in the unstimulated group, this decrease was approximately 1.9 times, and among hCG-stimulated males approximately 4.2 times. Also, after mating the concentration of testosterone in unstimulated males was about 2.9 times higher than in stimulated mink males. This would indicate clearly the effect of hCG stimulation on testosterone production. However, the present experiment does not show the pattern of changes in the concentration of the androgen. Perhaps a greater and/or possibly accelerated decline in testosterone is associated with better mating efficiency observed in hCG-stimulated males. However, drawing any further conclusions on the basis of the presented results would be speculation.

In conclusion, stimulation with doses of 100, 150 and 200 U hCG did not increase the number of mated females significantly. Small differences in favour of stimulated males have not been supported statistically. The results suggest that the

applied hCG stimulation procedure may not be optimal for male American mink, and therefore further research is needed to determine both the appropriate dosage of the hormone and the proper date of its administration. The results give reason to suspect that stimulation with hCG causes a more rapid decline in blood testosterone levels in male American mink.

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Wpływ stymulacji hCG na samce norki amerykańskiej (*Neovison vison*) w okresie kryć

STRESZCZENIE

Celem badań była ocena wpływu stymulacji jednorazową dawką hCG na libido u samców norki amerykańskiej w okresie kryć i na poziom testosteronu w ich krwi po zakończeniu sezonu kryć. Materiał badawczy stanowiły samce norki amerykańskiej. W grupie eksperymentalnej samcom podawano hCG w dawkach 100, 150 lub 200 U. Krew pobierano do analizy dwukrotnie, około 2 tygodnie przed okresem kopulacyjnym oraz po jego zakończeniu. W grupie otrzymującej 100 U hCG stwierdzono największy odsetek samców skutecznie kryjących samice w pierwszych 24 godzinach po stymulacji, natomiast najniższe libido w tym samym okresie wykazywały samce stymulowane dawką 200 U hCG. Z kolei samce z grupy otrzymującej dawkę 150 U hCG pokryły największą liczbę samic w całym okresie krycia. Średnie stężenie testosteronu w osoczu krwi u wszystkich badanych samców 18 lutego wynosiło 12,44 ng/ml. Spadek stężenia testosteronu po zakończeniu kryć był statystycznie istotny.