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INFLUENCE OF EXOGENOUS MELATONIN TREATMENT ON THE RAM FERTILITY OF THE NORTH-EAST BULGARIAN FINE-WOOL SHEEP BREED

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Abstract

The potential of the epiphyseal hormone melatonin to effect on seasonally breeding species has been extensively studied, but the data remain inconclusive. Levels of reproductive parameters modulation are important to monitoring, given the breed affiliation, as example analyzed breed, which has a pronounced seasonal type of reproduction. The objective of this study was to determine whether application of exogenous melatonin has an effect on ram fertility of the North-east Bulgarian fine-wool sheep breed, as estimated by the reproductive performances of ewes, in the non-breeding season. In addition, the study quantified the effects of exogenous melatonin on ejaculate volume and concentration of sperm in rams with melatonin implants ($n = 5$) and per os treated rams ($n = 5$). The application of exogenous melatonin improves the fertility of rams of the studied breed. As a result of the current study, in the 2nd experimental group there was a positive trend on ejaculate volume and concentration of sperm throughout the experiment compared to control group. Although rams exhibited an improvement in sperm parameters, a significantly increased birth rate was found in sheep mating with male receiving melatonin both through implants ($P < 0.05$) and per os ($P < 0.05$). It was found to increase the total percentage of birth lambs in 1st (28%) and 2nd (29%) experimental groups

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compared to control. The results are guiding for North-east Bulgarian fine-wool sheep farmers and breeders, in terms of opportunities to improve the breeding process and obtain more lambs.

Key words: ram, sperm, melatonin, North-east Bulgarian fine-wool sheep breed

Introduction. Sheep are seasonally breeding animals. Activation of the reproductive season is regulated by a surrounding photoperiod shortening, converted into a physiological signal by a change in the secretion of the pineal hormone melatonin [1, 2]. Thus, the use of exogenous melatonin is able to modify the reproductive performance of sheep by mimicking of the night prolongation. The degree of reproductive pause in the species is influenced by the climatic zone of breeding and varies between breeds, but nevertheless is more pronounced in female animals, manifesting itself in the cessation of ovarian function [3]. There is also a change in reproductive abilities in male animals, but less pronounced. Differences in behavioural and sperm parameters were observed during the non-breeding season, largely due to low testosterone levels during this period, but spermatogenesis did not stop. Melatonin corrects gonadal activity by influencing the hypothalamic-pituitary-gonadal axis and thus synchronizes reproductive activity with the surrounding seasonal changes. The hormone is able to indirectly affect testosterone concentrations in the blood [4, 5] by altering GnRH-induced gonadotropic secretion of FSH and LH [6, 7]. Exogenously administered hormone increased the size of the ram testicles [5, 8, 9], but there are conflicting data regarding its influence on the ejaculate volume produced and the functional sperm parameters [4]. There are also discrepancies as to when reproductive changes occur with respect to the timing of melatonin implantation. Currently, melatonin is used to influence the seasonality in the breeding process of sheep, and studies show that its use as an implant with extended release, pulls in advance the start of the breeding period in this species [8, 10].

The objective of this study was to determine whether application of exogenous melatonin (with implant or per os treatment) has an effect on ram fertility of the North-east Bulgarian fine-wool sheep breed, as estimated by the reproductive performances of ewes, in the non-breeding season. In addition, the study quantified the effects of exogenous melatonin on ejaculate volume and concentration of sperm.

Materials and methods. The study was conducted in Agricultural experimental station in Targovishte ($43°15'N 26°35'$E), at the end of May 2023. It included 15 rams of Northeastern Bulgarian fine-wool breed, maximally equal in weight and age. Animals were divided randomly into three groups (5 rams/group): control group, without addition of hormone; 1st experimental group, injected with melatonin implants; and 2nd experimental group, with melatonin intake with food (per os). The melatonin implants used in the 1st experimental group contained

18 mg melatonin (Melovine, Ceva Animal Health, France). Three implants per animal were placed subcutaneously behind the ear, using a specialized syringe gun, following as much as possible the principles of asepsis and observing the requirements for minimizing the stress of the manipulation. The rams from 2nd experimental group were individually supplemented with 5 mg melatonin (Natrol, USA). The hormone was added in the evening, according to natural increase in its endogenous levels. All animals were fed the same, following recommendations of the National Research Council [11] for sheep and also they had free access to water and mineral supplement. The rams were kept in individual pens 600 meters from the main flocks in order to avoid olfactory and sound stimulation from the sheep for at least two months before mating. The ejaculates were collected by artificial vagina three times – after a month, a month and a half, and at the end of experimental period – after 2 months from the melatonin application (June 19, July 10 and July 25). Sperm assessment was performed immediately after ejaculation in terms of volume (ml) and concentration $\times 10^9$ (cm^3). Determination of sperm count was performed after dilution, using a Burker counting chamber and in parallel CASA analyzer (Microptic, Spain).

To evaluate the effects of exogenous melatonin treatments on ram fertility, 416 sheep were mating with males from the three groups. At the end of the biological experiment we estimated pregnancy rate after first insemination of sheep (%), litter size (number of born lambs per group), fecundity (average number of lambs born per sheep). To obtain an objective measurement of the effects of the exogenous melatonin on fertility, we calculated the percentage of increase additional lambs born after mating with melatonin-treated rams (fecundity experimental gr. – fecundity control gr./fecundity experimental gr. $\times$ 100).

Statistical analysis. To determine whether the three groups differed in their response to the melatonin treatment, about other parameters, the groups were compared using a factorial analysis of variance. One-way ANOVA with Duncan's multiple range test was used to evaluate the significant differences at a level of 0.05.

Results. The results of the measured ejaculates volume showed non statistically significant differences ($P > 0.05$) among the groups of male animals during the control dates (Fig. 1). An increased ejaculate volume was found in the 2nd experimental group at the initial date. At the end of the experiment, the trend was reversed, animals that received melatonin per os, produced a smaller volume of semen than the control group ($P > 0.05$). Implanted rams secrete relatively equal volumes of ejaculate, with a tendency to increase at the end of the test period. In general, no effect on ejaculate volume can be claimed, but it can assume a partially suppressive effect of the hormone (Fig. 1).

Results of sperm concentration showed a trend towards higher sperm counts in the ejaculates of rams receiving oral melatonin, compared with both control

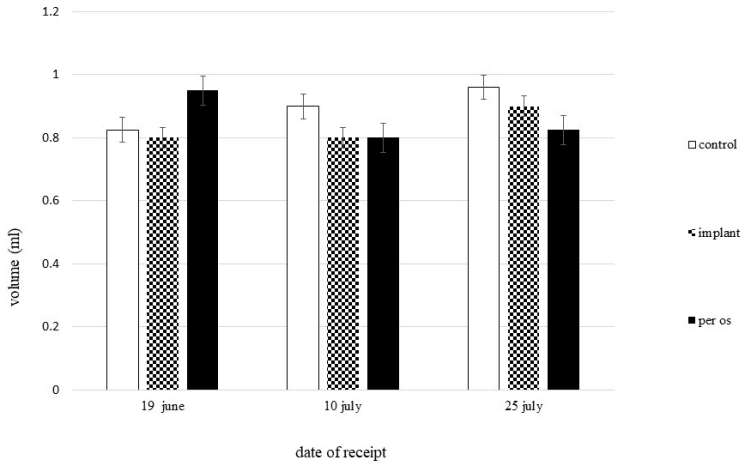

Fig. 1. Ejaculate volume (ml)

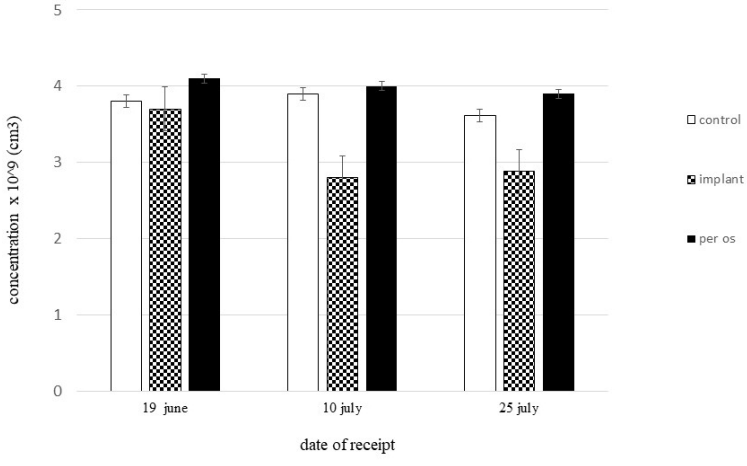

Fig. 2. Sperm concentration (cm^3)

and the 1st experimental group. The trend was maintained in all three semen collections (Fig. 2).

Despite discontinuation of melatonin, ejaculates from the 2nd experimental group continued to have higher positive trend of sperm concentrations than implanted rams of 1st experimental group. The results show that much of the effect is due to the central action of the hormone.

Data show an increase in the pregnancy rate from first insemination in experimental group compared with control (Table 1). A significantly increased fecundity was found per sheep mating with male receiving melatonin both through implants ($P < 0.05$) and per os ($P < 0.05$). The total percentage of birth lambs was found to increase in 1st (28%) and 2nd (29%) experimental groups compared to control.

Table 1

Effects of exogenous melatonin treatments on fertility of rams presented as pregnancy rate after first insemination of sheep (%), litter size (number), fecundity (mean ± SE)

Groups	Inseminated, n	Re-inseminated, n	Pregnancy rate from first insemination, (%)	Litter size, n	Fecundity (mean ± SE)
Control gr.	124	22	82.20	65	1.09 ± 0.07
1st exp. gr. (with implants)	218	31	85.78	144	1.52 ± 0.03 ($P = 0.004$)
2nd exp. gr. (melatonin per os)	74	12	84.62	48	1.54 ± 0.06 ($P = 0.009$)

Discussion. The practical questions related to the biology of reproduction of farm animals, in particular the species *Ovis aries*, highlight the need not only for informative sperm indicators, but also for extended in vivo research. The melatonin implants used cause elevated levels of the hormone in the bloodstream 24 hours a day. Thus, prolonging the time of melatonin signal induces the artificial short day and, accordingly, the beginning of the reproductive process. On the other hand, the administration of exogenous hormone only in the evening, during its natural increase in the body, would distinguish the effects of these 24-hour elevated levels, respectively, showing the positive and negative sides of both models of administration [12, 13]. Similar to the results in the current study about approximately equal sperm volume and concentration in both experimental groups (Fig. 1, 2), there was no difference in semen quality parameters in Bafra breed rams. However, exogenous melatonin in the non-breeding season significantly increased both testicular volume and testicular blood flow. On the basis of this observation, authors concluded that giving rams implants with 36 mg melatonin twice at least one month prior to the non-breeding season is expected to improve reproductive capacity [14]. Melatonin treatment can result in 40% more ejaculate doses in Ile de France breed rams than untreated males [10]. Melatonin treatment individually improves the percentage of motile and sperm with normal morphology in Konya Merino breed [5]. In our previous studies of the North-east Bulgarian fine-wool sheep breed, modulation of the motility and survival of their gametes was observed as a result of in vivo treatment with melatonin [15]. Other authors reported a decrease in sperm apoptosis-like characteristics and modulation of sperm capacitation as well as fertilization rates after in vitro melatonin treatment [16, 17]. It is believed that the effect is due to the antioxidant effect of melatonin, which as a result promotes the embryonic development [18]. This suggestion supports the obtained by us data about increasing the pregnancy rate by 3.58% in 1st experimental group and 2.42% from 2nd experimental group than the rate of sheep mated with control rams (Table 1). Data matches and comple-

ments the results of Abecia et al. [19]. According to the authors, the fertility of the treated sheep is improved and the number of lambs obtained is increased by 15–30% depending on the breed and time of implantation. The increased number of lambs born per ewe on Rasa Aragonesa (19%), Assaf (9%), and Manchega (7%) breed after mating with melatonin-treated rams [9] are responsive to our result – 28% and 29% more birth lambs in 1st and 2nd experimental groups, respectively.

The mechanisms by which melatonin improves reproductive activity are not yet fully understood, as the pineal hormone acts in various organs and tissues of the body. Apart from the effects at the hypothalamic-pituitary level, the facts about the local production of the hormone in the testicles are not insignificant. A melatonin effect at the ovarian level is also possible, responsible for delaying follicular atresia during folliculogenesis, leading to an increase in ovulation rate and probably improving fertility rate [16, 17, 19].

Conclusion. As a result of the current study, it was determined that the application of exogenous melatonin improves the fertility of rams of the North-east Bulgarian fine-wool sheep breed. In the 2nd experimental group there was a positive trend in ejaculate volume and concentration of sperm throughout the experiment compared to control group, also the two experimental groups did not differ significantly. Rams exhibited an improvement in sperm parameters and an increased birth rate was found in sheep mating with experimental rams receiving melatonin with implants (28%) or treated per os (29%) in comparison with control animals.

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