

## Melatonin significantly increases out of season lamb production in Blanc du Massif Central sheep

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### ABSTRACT

This practical study was the first to examine the effects of melatonin implants on the reproductive performance of Blanc du Massif Central ewes in Hungary. The ewes were divided into two experimental groups: group A (n=66) was treated with melatonin implants and group B (n=66) was the control group. Four parameters were measured in the two groups: fertility rates, the average number of lambs born, the proportion of twin pregnancies, and embryo mortality rates. Fertility rates, the average number of lambs born per ewe were significantly higher ( $P<0.01$ ) in group A (75.75% and 1.26 respectively) than in group B (36.36% and 0.23 respectively). The proportion of twin pregnancies was also significantly higher ( $P<0.05$ ) in group A (74.46%) than in group B (40.00%). Embryo mortality rate was significantly lower in group A (6.00%) than in group B (54.17%). Each group was further divided into three age groups (1–2 years, 3–5 years and 6–10 years old). In group A, there was also a significant increase ( $P<0.01$ ) in the average number of lambs born per ewe in age groups 1-2 years old (1.29) and 3-5 years old (1.47) in comparison with the respective age groups (0.21 and 0.19) in the control group. These results indicate that the use of melatonin in a continental area can improve the reproductive efficiency of ewes, but this effect may be age-dependent.

**Key words:** sheep; reproduction; melatonin; fertility; embryo mortality

### Introduction

Most European sheep breeds are seasonally polyoestrous, but some breeds are polyoestrous (year-round), with a short period of anestrus, usually during April and May. Their natural breeding season starts when the days become shorter and comes to an end when the days become longer ([THIMONI-](#)

[ER, 1981](#)). However, in the animal body, daylength is not measured by the total daily exposure to light, rather the illumination of two time periods of the day are important ([ORTAVANT et al., 1988](#)). This has an influence on the pineal gland and its melatonin secretion, or more precisely, the lack of illumination of these hours lifts the inhibition on melatonin output ([REITER et al., 2014](#)).

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Melatonin (N-acetyl-5-methoxytryptamine) is a hormone mostly produced by the pinealocytes in the pineal gland, and in other tissues to some extent (VINCE et al., 2017). The molecule has amphipathic characteristics and can penetrate almost any other tissue, such as the ovary in females or the testes in males (AMARAL and CIPOLLA-NETO, 2018; ŽURA ŽAJA et al., 2018). It helps to regulate physiological functions such as circadian rhythm, immunity, neuroprotection and reproduction (ŽURA ŽAJA et al., 2020; BOUROUTZIKA et al., 2021; SHABAJEE-ALIBAY et al., 2022). These activities can be divided into two categories: receptor mediated and receptor independent actions (REITER et al., 2014). Receptor mediated actions, such as affecting reproduction, happen mostly through the MT1 and MT2 receptors, which can also be found in cumulus cells, oocytes, and Sertoli-cells. This indicates that melatonin plays a key role in follicle growth, oocyte maturation, and testosterone production in sheep (XIAO et al., 2019; POOL et al., 2020). Melatonin is a potent antioxidant through its receptor independent action, where it directly detoxifies reactive nitrogen species (RNS) and reactive oxygen species (ROS) (REITER et al., 2014). These characteristics were also examined in other *in vitro* studies, which showed that melatonin helps the maturation of oocytes and the embryo, and can protect endometrial epithelial cells, potentially increasing litter size. However, the exact mechanism remains unclear of how, while acting as a trigger for the start of the neuroendocrine regulation of reproduction, melatonin treatment alters the production and effects of the follicle stimulating hormone (FSH) in a way that does not increase the amount of triplet litters during natural breeding (TIAN et al., 2017; GE et al., 2022; YAO et al., 2022).

By applying these characteristics in practice, melatonin can improve the reproductive performance of ewes and rams during out of season mating periods by mimicking the effect of shorter days in Mediterranean regions (PULINAS et al., 2021).

Furthermore, melatonin can help improve the redox status and milk yield of ewes under stress, and reduce embryo mortality rate in recipient ewes after embryo transfer (SONG et al., 2019; BOUROUTZIKA et al., 2020).

The aim of this practical study was to determine the effect of melatonin implants on the reproductive performance of Blanc du Massif Central (BMC) sheep during the spring mating period in Hungary, and whether animal age had any effect on reproduction.

### Materials and methods

The study was conducted on a BMC conventional sheep breed flock in Hungary. On the farm, the production method is based on three lambings over two years, with natural breeding. There are three mating periods in each year, one in April–May, one in August–September and the last in December–January. Ewes participate in every second mating season. Due to the unfavourable weather during April–May, ewes have poorer fertility rates (35±3%) than in August–September (60±4%) or December–January (50±3%) mating periods. This study was conducted during the April–May mating season.

In total, 152 ewes and 4 rams were included in the experiment, accommodated in a semi-open barn with fresh straw bedding. Animals with a body condition score (BCS) between 2.5–3 (where, 1: pathological underweight; 2: underweight; 3: medium; 4: obese; 5: pathological obese) were selected and checked for any clinical sign of infection or illness, and the ewes were divided into two groups:

Group A: 66 ewes (n=66) treated with melatonin implants (Melovine® 18 mg Ceva Sante Animale, France) according to the manufacturer's instructions.

Group B: 66 ewes (n=66) as the control group, where melatonin treatment was not used.

Both groups were further divided into three age groups:

1–2 years:  $n_{\text{group A}}=28$ ;  $n_{\text{group B}}=33$

3–5 years:  $n_{\text{group A}}=30$ ;  $n_{\text{group B}}=26$

6–10 years:  $n_{\text{group A}}=8$ ;  $n_{\text{group B}}=7$ .

In the experiment, 3–5 years old rams with known fertility were chosen. All four rams had similar fertility results during the two mating seasons preceding the experiment. The rams showed no signs of illness prior to or during the mating season. Rams were only used as breeding males and were not part of the statistical analysis.

During the experiment, all animals were fed with hay (metabolisable energy: 7.9 MJ/kg dry matter, crude protein: 88 g/kg dry matter) and water *ad libitum*, and 0.5 kg barley (metabolisable energy: 12.4 MJ/kg dry matter, crude protein: 124 g/kg dry matter) per animal was added to the feed each day.

Rams and ewes were kept together only during the mating period, which lasted from 19 April 2023 to 29 May 2023 (41 days in total). At 40 days after the end of the mating period, the ewes were checked for gravidity using a Dрамиński® Animal Profi 2 ultrasound machine with a Dрамиński® 3–7 MHz mechanical abdominal probe (Dрамиński S. A. Poland). The ultrasound pregnancy test was carried out by positioning the probe at a 90-degree angle to the surface of the right abdominal region of the animal, in front of the udder, 10–15 cm from the *linea alba*.

Fertility rates in both groups were calculated for each group on the basis of the results of the ultrasound test, and the average number of lambs born per ewe, the ratio of twins born, and embryo mortality rate were recorded.

Fertility rates were calculated from the positive and negative results of the ultrasound test by dividing the positive results by the total number of ewes in each group. The average number of lambs per ewe was calculated per group, where ewes that had no lambs were given a value of zero lambs. The calculation was done by dividing the total number of lambs born in each group by the respective total number of ewes. In the comparison of the ratio of twin litters, only double twins were compared and triplet litters were included in the twin category. The calculation was done by dividing the number of twin litters in each group by the respective total number of litters in the group. Embryo mortality rate was calculated by dividing the number of ewes that scanned positive for pregnancy without lambing,

compared to the number of ewes scanned for positive pregnancy that gave birth to at least one lamb. No abortions were recorded during the experiment

For statistical analysis we used the R program version 4.1.3. To compare the fertility rates, the ratio of twin litters and the embryo mortality rate between the groups was analysed using the two-way contingency table with Pearson's chi-squared test, and ANOVA models were used to compare the average number of lambs born per ewe.

## Results

**Fertility rates.** In group A, 50 of 66 ewes were positive for pregnancy (75.75%), while in group B 24 of 66 were pregnant (36.36%), as presented in Fig. 1. The fertility rates divided by age in group A were the following: 1–2 years: 21 of 28 ewes (75%), 3–5 years: 25 of 29 ewes (86.20%), 6–10 years: 4 of 8 ewes (50.00%) were pregnant. In group B, fertility rates in each age group were the following: 1–2 years: 11 of 33 ewes (33.33%), 3–5 years: 9 of 26 ewes (34.61%), 6–10 years: 4 of 7 ewes (57.14%) were pregnant.

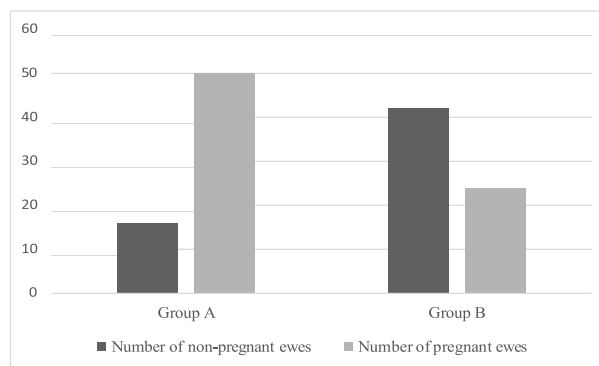


Fig. 1. Fertility rates in group A (melatonin treatment) and in group B (control)

The average number of lambs born per ewe. In group A, the average number of lambs born per ewe was  $1.26 \pm 0.89$ , as compared to  $0.23 \pm 0.60$  in group B, which is presented in Fig. 2.

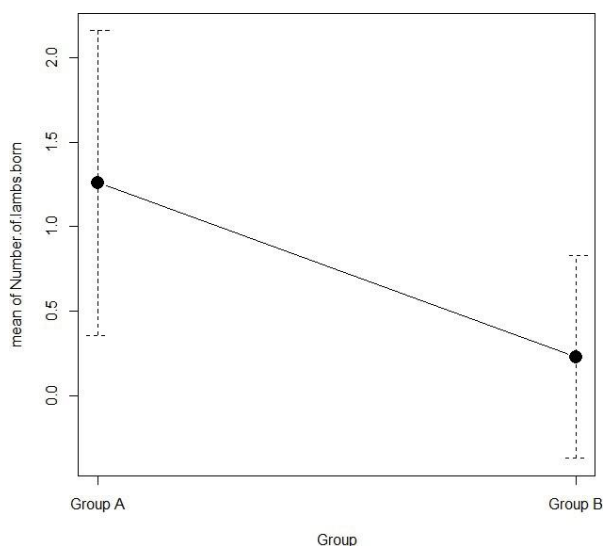


Fig. 2. The average (mean) number of lambs born per ewe in group A and group B

The mean number of lambs born per ewe by age groups are presented in Table 1.

*The ratio of twin litters from lambed ewes.* In group A, there were 35 twin and 12 non-twin litters (74.46%) and this was more than in group B, in which there were four twin and six non-twin litters (40.00%). These results are shown in Fig. 3.

*Embryo mortality rate.* Embryo mortality was recorded in three ewes that did not lamb out of 50 pregnant ones (6.00%) in Group A, which was lower than in group B, in which 13 ewes did not lamb out of 24 pregnant ones (54.17%), as presented in Fig. 4.

Table 1. The average number of lambs born per ewe and the total number of ewes in each experimental and age group

Group	Average number of lambs born per ewe		Number of ewes	
	Group A	Group B	Group A	Group B
<b>Overall</b>	1.26 <sup>a</sup>	0.23 <sup>a</sup>	66	66
<b>1-2 years</b>	1.29 <sup>b,d1</sup>	0.21 <sup>b</sup>	28	33
<b>3-5 years</b>	1.47 <sup>c,d2</sup>	0.19 <sup>c</sup>	30	26
<b>6-10 years</b>	0.38 <sup>d3</sup>	0.42	8	7

Letters in the superscript indicate significant differences (a-a; b-b; c-c; d1-d3; d2-d3)

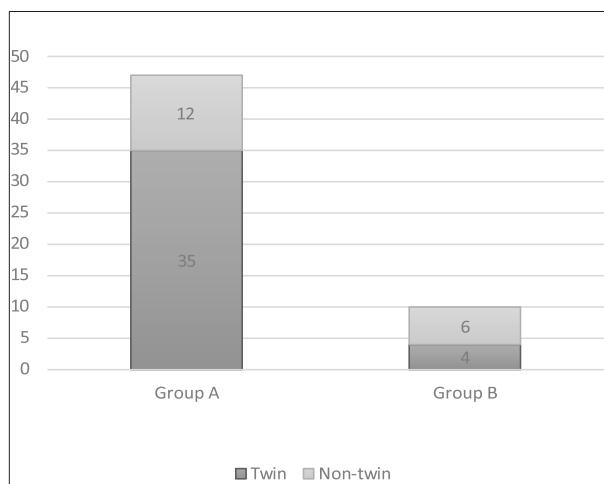


Fig. 3. The proportion of twin litters in group A (melatonin treated) and group B (control)

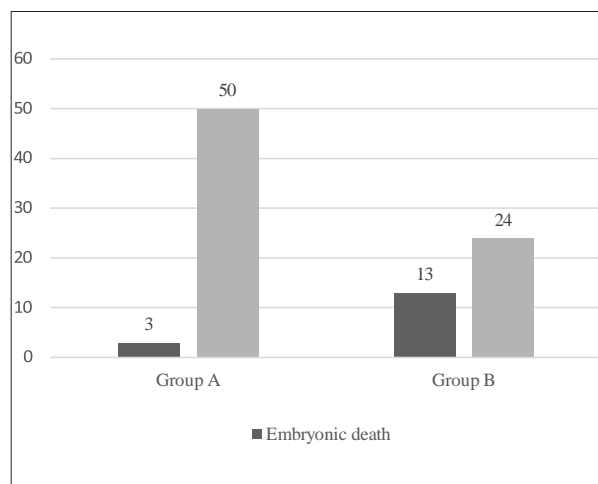


Fig. 4. Embryo mortality rate in group A (melatonin treated) and in group B (control)

## Discussion

There was a significant difference ( $P < 0.01$ ) in the fertility rates between the groups, indicating that exogenous melatonin treatment improved the reproductive performance of the ewes. This was in line with previous findings ([SCHOEMAN and BOTHA, 1995](#); [LURIDIANA et al., 2015](#); [CEVIK et al., 2017](#); [MURA et al., 2019](#); [ADNANE and MIROUD, 2024](#)). It is also important to note that in group A, the differences in fertility rates were significant between age groups ( $P < 0.05$ ), with the 6–10 year group having the lowest rates, although this difference was not detected in group B. This was contrary to previous reports in Sardinia ewes, where 5–6 year olds showed a better response to melatonin treatment than 3–4 year old ewes, or untreated ewes ([LURIDIANA et al., 2015](#)).

The average number of lambs born per ewe was significantly higher ( $P < 0.01$ ) in group A than in group B. We used this term instead of litter size, since it gives a better overview of reproductive performance, due to the involvement of ewes that did not lamb. This aspect can also be crucial from the perspective of flock management and farm economy. During the analysis, we observed that the difference was mainly in the younger age groups (1–2 and 3–5 years,  $P < 0.01$ ), and that there was almost no difference between the 6–10 year groups. Although this indicates that the reproductive performance of ewes cannot be further enhanced significantly by melatonin over the age of 5 years, since the number of ewes in the 6–10 year age group was small, further studies with melatonin should be carried out to be able to draw firm conclusions. This result also suggests the examination of other types of oestrus induction methods in this subgroup, since this may be an important factor for deciding the culling age for animals. Ewes without stable offspring production that cannot be further enhanced due to age, but consume the same amount of feed as others, will not only produce a deficit for farmers, but may also be more prone to obesity, causing animal health issues and further expenditures ([KALYESUBULA et al., 2020](#); [RASMUSSEN et al., 2024](#)).

Melatonin administration in ewes also significantly increased the number of twin litters ( $P < 0.05$ ), without increasing the number of triplets. However,

this may be favourable, since ewes with triplet litters need more, higher-quality feed than singlet or twin-bearing ewes ([DØNNEM et al., 2020](#)). Triplet lambs are also born with a lower bodyweight and cannot suckle simultaneously. These traits of triplet litters make them less desirable on farms with lower management levels, because if farmers cannot give these ewes and lambs the necessary treatment, the vitality of both ewes and lambs will decline ([DØNNEM et al., 2020](#)).

The embryo mortality rate was significantly lower in the treated group than in the control ( $P < 0.05$ ), which may have been caused by the free scavenging and antioxidant traits of melatonin. Similar results were reported earlier in recipient and donor ewes, where melatonin improved the survival rate of sheep embryos in recipient ewes ([SONG et al., 2019](#)).

These results showed that melatonin can improve the efficiency of the reproductive performance of sheep farms. The importance of these findings for sheep farming has several aspects. First, even in year-round polyoestric sheep breeds, the productivity of a spring or early summer (out of regular season) mating period can be inferior to an autumn or early winter mating period in continental regions. For this comparison, we used the results of a previous study conducted in the same herd ([LAKATOS et al., 2023](#)).

## Conclusions

This was the first study in Hungary to examine the effects of melatonin implants on the reproductive performance of sheep. These results showed that melatonin implants in BMC ewes could greatly increase the fertility rate, the average number of lambs born per ewe, and the ratio of twin litters, meanwhile decreasing the embryo mortality rate in the out of season mating period, although these results were not pronounced in older animals. This would result in a higher number of lambs born per ewe, which could ultimately improve the economic perspectives of farms.

### Ethics statement

The study was authorised under the HB/15-ÉLB/00 136-18/2023 permit number.

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#### **SAŽETAK**

Istraživanje je provedeno u praksi kako bi se procijenilo učinke implantata melatonina na reproduktivnu učinkovitost ovaca pasmine Blanc du Massif Central uzgajanih u Mađarskoj. Ovce su bile podijeljene u dvije pokusne skupine: skupinu A (n=66), koja je tretirana melatoninskim implantatima, i skupinu B (n=66), koja je služila kao kontrolna skupina. U obje su skupine mjerena četiri pokazatelja: stopa plodnosti, prosječan broj ojanjene janjadi, udio blizanačkih graviditeta i stopa embrionalne smrtnosti. Stope plodnosti i prosječan broj ojanjene janjadi po ovci bili su znakovito veći ( $P<0,01$ ) u skupini A (75,75% i 1,26) nego u skupini B (36,36% i 0,23). Udio blizanačkih graviditeta također je bio znakovito veći ( $P<0,05$ ) u skupini A (74,46%) u usporedbi sa skupinom B (40,00%). Stopa embrionalne smrtnosti bila je znakovito manja u skupini A (6,00%) nego u skupini B (54,17%). Svaka je skupina ovaca dodatno podijeljena u tri dobne skupine (1–2 godine, 3–5 godina i 6–10 godina). U skupini Azabilježeno je i znakovito povećanje ( $P<0,01$ ) prosječnog broja ojanjene janjadi po ovci u dobnim skupinama od 1 do 2 godine (1,29) i 3–5 godina (1,47) u usporedbi s odgovarajućim dobnim skupinama u skupini B (0,21 i 0,19). Dobiveni rezultati upućuju na to da primjena melatonina u kontinentalnim područjima može poboljšati reproduktivnu učinkovitost ovaca, ali taj učinak možda ovisiti o dobi.

**Ključne riječi:** ovce; reprodukcija; melatonin; plodnost; mortalitet embrija

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