

DOI

Short communication

# Effects of melatonin treatment on twin pregnancy and fetal mortality in sheep

Gy. Lakatos<sup>1</sup>, B.N. Vincze<sup>2</sup>, N. Vass<sup>3</sup>, A. Dobos<sup>4</sup>

<sup>1</sup> University of Veterinary Medicine, Budapest Doctoral School, 1078 Budapest, István u. 2, Hungary

<sup>2</sup> University of Veterinary Medicine,

Budapest Department of Obstetrics and Food Animal Medicine, 1078 Budapest, István u. 2, Hungary

<sup>3</sup> University of Debrecen Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature, 4032 Debrecen, Böszörményi út 138, Hungary

<sup>4</sup> Large Animal Clinical Laboratory, University of Veterinary Sciences, Faculty of Veterinary Medicine, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

Correspondence to: Gy. Lakatos, email: osturul95@gmail.com

## Abstract

This study is the first to examine the effects of melatonin implants on the litter size and fetal mortality rate of Blanc du Massif Central ewes in Hungary. The ewes were divided into two experimental groups: group A: 96 ewes were treated with melatonin implants and group B: 114 ewes was the control group. The number of twin litters was significantly larger in group A than in group B, whereas the fetal mortality rate was significantly lower in the melatonin treated group than in the control group. These findings suggest that melatonin implants may provide significant benefits for sheep production in continental regions; however, their application requires careful consideration due to potential adverse effects on flock management associated with an increased incidence of twin pregnancies.

**Keywords:** fetal mortality, melatonin, reproduction, sheep, twin pregnancy

## Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone found naturally in animals (including humans). Most of this molecule is produced by the pineal gland and can penetrate almost any tissue, such as the ovary in females because of its amphipathic characteristics (Amaral 2018). Melatonin helps to regulate several

physiological functions for example neuroprotection, immunity, circadian rhythm and reproduction (Bouroutzika et al. 2021).

Although the secretion of melatonin is a complex neuro-endocrine process, and this hormone has various roles, its route of actions can be divided into two major categories: receptor-mediated and receptor independent ones (Reiter et al. 2014). Reproduction is directly



affected by receptor-mediated actions through the MT1 and MT2 receptors, which can also be found in cumulus cells and oocytes (Xiao et al. 2019). Melatonin is also a potent antioxidant through receptor independent actions, by directly eliminating reactive nitrogen (RON) and reactive oxygen species (ROS) which indirectly helps the maturation of oocytes and protects endometrial epithelial cells (Reiter et al. 2014, Tian et al. 2017).

These characteristics of melatonin can help to improve the reproductive performance of sheep by mimicking the effect of short days, improving the redox-status of animals and reducing embryo mortality rate in recipient ewes after embryo transfer (Song et al. 2019).

The aim of this study was to determine whether melatonin can increase litter size and reduce fetal mortality rate in Blanc du Massif Central (BMC) sheep in Hungary.

## Materials and Methods

The study was conducted in a conventional BMC sheep farm in Hungary from the 1<sup>st</sup> of March 2023 to the 31<sup>st</sup> of August 2024. In total there were 210 ewes and 6 rams in the experiment. All of them were accommodated in a semi open barn with fresh straw bedding. Animals with a body condition score of between 2.5-3 (where 1: pathologically underweight; 2: underweight; 3: medium; 4: obese; 5: pathologically obese) were selected and checked for any sign of infection, illness and reproductive deficiency. The ewes were randomly allocated to two groups:

Group A: 96 ewes (n=96) treated with melatonin implants (Melovine® 18 mg Ceva Sante Animale, France) 45 days prior to ram introduction according to the manufacturer's instructions.

Group B: 114 ewes (n=114) as the control group, where 0.2 ml sterile isotonic saline was used as placebo.

In the experiment, 3–5 year-old rams with known fertility were chosen. All six 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 experiment.

The mating period in each year – 2 periods in total – started on the 19<sup>th</sup> of April and lasted for 41 days. The rams were introduced to the ewes at the starting day of both spring (2023 and 2024) mating periods. At 40 days after the end of each mating period, the ewes were checked for gravidity using a Dramiński© Animal Profi 2 ultrasound machine with a Dramiński© 3-7 MHz mechanical abdominal probe (Dramiński S. A. Poland).

The ratio of twin litters was calculated by dividing the number of twin litters in each group by the respective total number of litters in the group. Those litters in which two or more lambs were born were classified as twin litters. Fetal mortality rate was calculated by dividing the number of ewes scanned positive for pregnancy without lambing, compared to the number of ewes scanned for positive pregnancy and which 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., binomial test.

## Results and Discussion

### Ratio of twin litters

In the melatonin group there were 43 twin litters and 25 single ones (63.24 %) and in the control group there were 14 and 14 respectively (50.00 %) (Fig. 1.). The difference between the melatonin group and control group was significant ( $p \leq 0.05$ ) with a 95 % confidence interval of 0.5067 to 0.7461. There were 3 triplet litters in the melatonin group and one triplet litter in the control group, but these numbers were too small to perform a dependable statistical analysis. This difference between the melatonin group and control group is in correspondence to previous findings, that melatonin implants can increase litter sizes (Scott et al. 2009). Although this might increase the productivity of the farm, the lambs in twin litters generally have lower body weight at birth, show lesser growth until weaning, and are prone to having more percentage body fat than singletons, if ewes are not fed adequately during early gestation (Kelman et al. 2022). Male lambs from twin litters also show worse reproductive performance than singleton ones at their first mating season, which can be an important factor in intensive farming systems (Sánchez-Dávila et al. 2021).

### Fetal mortality

In the melatonin group there were 72 observed pregnancies from which 68 resulted in lambing and in 4 cases fetal mortality was observed. In the control group there were 47 observed pregnancies from which 28 resulted in lambing and in 19 cases fetal mortality was observed. This difference between the two groups were significant ( $p \leq 0.01$ ) with a 95 % confidence interval of 0.0153 to 0.1362. This might be the result of the antioxidant effect of melatonin and similar results were observed in recipient ewes previously, in which case melatonin improved the survival rate of transferred sheep embryos (Song et al. 2019).

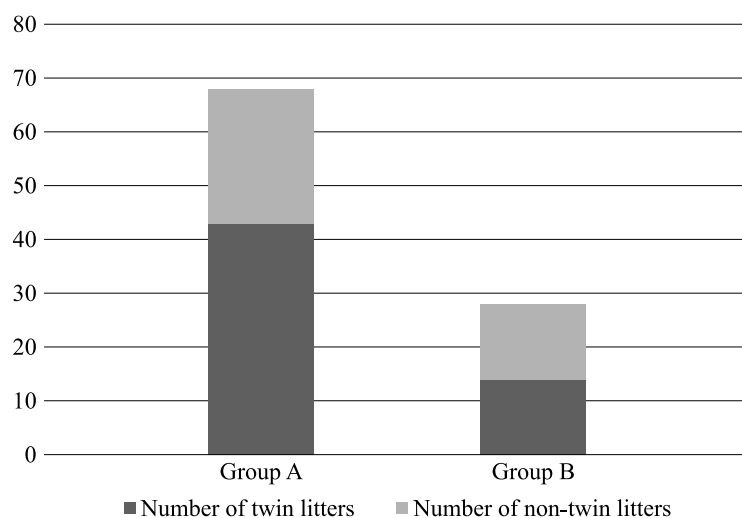


Fig. 1. Ratio of twin and non-twin litters in melatonin treated (Group A) and control (Group B) Blanc du Massif Central ewes.

## Conclusion

Melatonin is a multi-effect hormone which can reduce the fetal mortality rate and increase the litter size in sheep during breeding seasons. However, lambs originating from twin pregnancies require greater time and resource allocation during early growth, which should be accounted for when considering the administration of melatonin implants.

## Author Declarations

### Ethics approval

This study was authorised under the HB/15-ÉLB/00136-18/2023 permit number.

### Use of generative artificial intelligence

The authors did not use generative artificial intelligence (AI) tools in the preparation of the manuscript.

### Conflict of interest

The authors declare that they have no known competing interest.

## References

Amaral FGD, Cipolla-Neto J (2018) A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metab* 62: 472-479.

- Bouroutzika E, Ciliberti MG, Caroprese M, Theodosiadou E, Papadopoulos S, Makri S, Skaperda ZV, Kotsadam G, Michailidis ML, Valiakos G, Chadio S, Kouretas D, Valasi I (2021) Association of Melatonin Administration in Pregnant Ewes with Growth, Redox Status and Immunity of Their Offspring. *Animals* 11: 3161.
- Kelman KR, Alston-Knox C, Pethick DW, Gardner GE (2022) Sire Breed, Litter Size, and Environment Influence Genetic Potential for Lamb Growth When Using Sire Breeding Values. *Animals* 12: 501.
- Reiter RJ, Tan DX, Galano A (2014) Melatonin: Exceeding Expectations. *Physiology (Bethesda)* 29: 325-333.
- Sánchez-Dávila F, Lombardo J, Freitas-de-Melo A, Barragán HB, Ungerfeld R (2021) Singleton or twin male lambs: effects on their reproductive development. *Anim Reprod Sci* 231: 106797.
- Scott PR, Sargison ND, Macrae AI, Gough MR (2009) Melatonin treatment prior to the normal breeding season increases fetal number in United Kingdom sheep flocks. *Vet J* 182: 198-202.
- Song Y, Wu H, Wang X, Haire A, Zhang X, Zhang J, Wu Y, Lian Z, Fu J, Liu G, Wusiman A (2019) Melatonin improves the efficiency of super-ovulation and timed artificial insemination in sheep. *PeerJ* 7: e6750.
- Tian X, Wang F, Zhang L, He C, Ji P, Wang J, Zhang Z, Lv D, Abulizi W, Wang X, Lian Z, Liu G (2017) Beneficial Effects of Melatonin on the In Vitro Maturation of Sheep Oocytes and Its Relation to Melatonin Receptors. *Int J Mol Sci* 18: 834.
- Xiao L, Hu J, Song L, Zhang Y, Dong W, Jiang Y, Zhang Q, Yuan L, Zhao X (2019) Profile of melatonin and its receptors and synthesizing enzymes in cumulus – oocyte complexes of the developing sheep antral follicle – a potential estradiol-mediated mechanism. *Reprod Biol Endocrinol* 17: 1.