Short communication
DOI: /10.5513/JCEA01/20.1.2429
Journal of Central European Agriculture, 2019, 20(1), p.62-67

Reproductive activity in sheep with different lambing period treated with melatonin in April

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Abstract

The object was to evaluate the effect of melatonin treatment on the advance in April of the reproductive resumption in Sarda breed sheep with different lambing period. For the research two farms, located in North Sardinia between 39° and 40° N, were chosen. In each farm, 120 lactating ewes were selected: 30 lambed between October 20th and November 20th (group 1); 30 lambed between December 1st and 30th (group 2); 30 lambed between January 1st and 30th; 30 lambed between February 1st and 28th (group 4). In each farm, each group of 30 animals was divided into two subgroups of 15 animals (M and C). On April 1st, in each farm, the animals of the M subgroups were treated with a implant containing 18 mg melatonin. The subgroups C were kept as control. The lambing dates and the number of newborn lambs were recorded until 220 days after ram introduction. In treated animals greatest fertility ($P<0.01$) and lowest distance in days from male introduction to lambing ($P<0.01$) were recorded. The best reproductive performances were found in the group 1 and 2 compared to the other two groups ($P<0.01$).The present research shows that melatonin treatment should be made 3 or 4 months after lambing, in order to obtain optimal results.

Keywords: fertility rate, lambing date, melatonin, Sarda sheep breed

Introduction

Sheep at the Mediterranean latitudes show a reproductive seasonality linked to the photoperiod pattern (Mura et al., 2014). The perception of light/dark alternation causes the variation of melatonin secretion by the epiphysis, with high levels at night and low levels during the hours of light (Carcangiu et al., 2014). Melatonin is considered as the organic informer of the photoperiodic trend, and then the regulator of reproductive seasonality. The placement of melatonin micro implants in close proximity to some hypothalamic nuclei stimulates the luteinizing hormone (LH) secretion (Malpaux et al., 1998). The melatonin administration showed to improve the

reproductive efficiency in different sheep breeds, and subcutaneous implants are the most widely used in Europe and in other continents (Chemineau et al., 1996; de Nicolo et al., 2009; Luridiana et al., 2015). The melatonin released by the implants is able to mimic short days, without causing an inhibition of pineal secretion of melatonin, so stimulating reproductive activity in sheep (Staples et al., 1992). At Mediterranean latitudes, in dairy sheep, the advance of lambing at the beginning of Autumn is an essential requirement for a duration of 6-7 months of the lactation (Carcangiu et al., 2012). Thus, in order to allow Autumn lambings, sheep should reproduce in spring, during their anoestrus period. Moreover, in Spring dairy sheep, such as the Sarda breed, are lactating and then it determines that their energies are primarily used for milk production rather than for other physiological activities such as reproduction (Carcangiu et al., 2012). In Sarda breed sheep, in fact, the reproductive response to melatonin treatment is not always optimal and many times the fertility rates result equal between treated and controls (Mura et al., 2010). Therefore, the effect of the melatonin could be masked by other factors (Luridiana et al., 2015) such as lambing date. Thus, the object of the present research was to evaluate the effect of treatment with melatonin on the advance in April of the reproductive resumption in Sarda breed sheep with different lambing period.

Material and methods

For the research two farms located in North Sardinia between 39° and 40° N, in the same climatic and altitude area, and with the same nutritional and management regime, were chosen. Each farm raised about 800 Sarda sheep and the animals were maintained under natural photoperiod since birth. During the day the animals grazed on leguminous and gramineous grasses, also they received 300 g per head daily of concentrate commercial food (crude protein 20.4% and 12.5 MJ ME/kg DM) at the time of milking. In each farm, 120 lactating ewes were selected (30 lambed between October 20th and November 20th (group 1); 30 lambed between December 1st and 30th (group 2); 30 lambed between January 1st and 30th (group 3); 30 lambed between February 1st and 28th (group 4). The chosen sheep were 3 to 6 years old and they had a Body Condition Score (BCS) between 3.0 and 4.0. The number of the ruminal bolus of each animal was recorded, and the ewes were individually marked with numbered collars to avoid recognition errors. In each farm (identified as F1-F2) the chosen 120 ewes were kept away from the rest of the flock. The BCS was detected on 5th April, date of ewes' selection, and after one month, according to the methods by Russel et al. (1969). The scores ranged from 1 (very poor condition) to 5 (very good condition) in half-unit increments. Scoring was based on the results of feeling the amounts of muscling and fat deposition over and around the vertebrae in the loin region. In each farm, each group of 30 animals was divided into two subgroups of 15 animals (M and C). On April 1st, in each farm, the animals of the M subgroups were treated with a slow release implant containing 18 mg melatonin, in the left retroauricular region. The subgroups C were untreated and kept as control. In every group of 30 ewes, on May 5th, 2 males of proven fertility were introduced. The rams were removed from females after 70 days of cohabitation, so that the unmated ewes at this date remained empty. Gestation was diagnosed by transabdominal ultrasonography examination using Esaote Piemedical Tringa linear equipment (Esaote Europe B.V., Maastricht, the Netherlands) equipped with 5 – 7.5 MHz

multiple frequency linear probe. Pregnancy diagnosis has been performed in all sheep, every week, from 45 days after male introduction to 45 days after the male removal from the groups. The lambing dates and the number of newborn lambs were recorded until 220 days after ram introduction.

R statistical software, Version 3.2.2 (R Development Core Team, 2011) was used to perform the statistical analysis. A General Linear Model (GLM) procedure was performed to analyse the effect of lambing period and treatment on the litter size and on the distance in days from ram introduction to lambing. To compare percentages of lambed ewes within each lambing period chi-square test was used. A P value<0.05 was considered statistically significant.

Results and discussion

The effect of melatonin treatment on the advance of the reproductive resumption in all groups is clearly shown by the average of days from male introduction to parturition compared to controls. In fact, greater fertility (P<0.01) and less distance in days from male introduction to lambing (P<0.01) were recorded in treated animals, compared to controls (Table 1). Moreover, the treated animals have an advance of 10 days in lambing date compared to controls.

Table 1. Fertility rate, distance in days from ram introduction to parturition and litter size according to Groups (M or C) in Sarda breed sheep

Group	Subgroup	Animals	Fertility rate (%)	DIML (days)	Litter size
1	M	30	85^{B}	174.1 ± 15.1^{A}	1.18
	C	30	73^{A}	185.4 ± 18.3^{B}	1.21
2	M	30	87^{B}	178.6 ± 16.2^{A}	1.19
	C	30	72^{A}	187.9 ± 15.4^{B}	1.25
3	M	30	70^{B}	188.4 ± 18.9^{A}	1.22
	C	30	61^{A}	196.5 ± 14.8^{B}	1.24
4	M	30	52^{B}	194.2 ± 17.5^{A}	1.19
	C	30	40^{A}	203.6 ± 18.1^{B}	1.14

DIML - distance in days from male introduction to lambing. The statistical differences between treated and control subgroups are related within the group to which they belong; ^{A,B} P<0.01.

Furthermore, the best reproductive performances were found in the animals that lambed in October-November and December compared to the other groups. The litter size showed no variations among groups. The treated animals of the groups 1 and 2 showed the lambing peak 160-170 days after the male introduction, while in the controls the peak is recorded between 170 and 180 days. In the 3M subgroup the lambing peak is recorded between 180-190 days, and in the 4M subgroup between

190-200 days after male introduction. In the 3C and 4C subgroups there is no real lambing peak.

Then, in this study, treated animals responded earlier to the ram effect showing a more compact mating and subsequently lambing period, than untreated. This trend is in agreement with that found by Abecia et al. (2006) which observed an earlier conception pattern and a more concentrated lambing period in a melatonin-treated group than in an untreated control group. Moreover, melatonin treatment induced a significant improvement in fertility rate in M subgroups showing a higher number of lambed ewes than in subgroups C. The improved fertility in the treated ewes could be due to melatonin effect not only on the secretion of GnRH and LH, but also directly on ovarium (Abecia et al., 2006; Tamura et al., 2009). Indeed, the melatonin receptors are present in different ovarian structures where this hormone produces a higher number of ovulatory follicles, a minor atresia and is involved in improving oocyte maturation and luteal function (Yie et al., 1995; El-Raey et al., 2011; Tian et al., 2017). Furthermore, melatonin could also have a protective effect against free radicals, favouring the follicles growth (Tamura et al., 2014). The lowest fertility observed in ewes of groups 3 and 4, both treated and control, are certainly related to the metabolic-hormonal status typical of the lactation. Indeed, these animals were in the stage of high milk yield and the resources of the organism were used for the milk secretion (Carcangiu et al., 2012). This also explains the different results obtained with the treatment of melatonin in the Sarda sheep in previous study.

Conclusions

In conclusion, data confirm the positive effect of melatonin treatment on the reproductive resumption in Sarda sheep. Furthermore, the present research shows that melatonin treatment should be made 3 or 4 months after lambing, in order to obtain optimal results.

Acknowledgements

This research was supported by grants from the RAS, project entitled MIGLIOVIGENSAR.

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