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# Melatonin treatment in winter and spring and reproductive recovery in Sarda breed sheep

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ABSTRACT

This study aimed to evaluate the effect of melatonin treatment carried out between late winter and early spring on reproductive response in Sarda breed sheep and whether the photo-refractoriness can influence this reproductive response. The study was conducted on 3200 adult ewes, aged 3–6 years old, with body condition score (BCS) 2.5–4.0, from 16 commercial sheep farms in Northern Sardinia. In each farm 200 animals were enrolled and subdivided into 2 groups (n = 100 each): Group M (treated with one 18 mg melatonin implant), and group C (untreated). The melatonin treatments were performed on 10th February; 10th March; 10th April and on 10th May each time in 4 different randomly selected farms. Adult males, treated with 3 melatonin implants 1 week before females, were introduced in each flock 35 days after ewes' treatment, and removed after 45 days of cohabitation with females. Pregnancy was determined by transabdominal ultrasonography examination between 45th and 90th day after ram introduction. Data showed that melatonin treatment increased the fertility rate significantly ($P < 0.05$), with the higher fertility rate in the ewes treated in April and May. The average time in days from male introduction to lambing was shorter in treated than in control ewes ($P < 0.05$). Further, at the 160th and 170th day after male introduction the group M showed a higher number of lambed ewes compared to C ($P < 0.01$). This effect was observed at 180th and 190th days after ram introduction, also, but with lower significance ($P < 0.05$). In conclusion, melatonin treatment improved reproductive efficiency and advanced breeding season in Sarda sheep, especially when ewes were treated in spring.

## 1. Introduction

Reproductive seasonality of dairy sheep at the Mediterranean basin latitudes is a major constraint for production (Chemineau et al., 2007). Furthermore, these areas have a mild climate during winter and autumn, rainfall mainly concentrated in spring and a very dry summer. Thus, the sheep production cycle is closely linked to pasture growth which occurs predominantly in autumn and spring (Carcangiu et al., 2011). Therefore, to increase the length of lactation (6–7 months), it is necessary to mate sheep in spring to obtain lambing in autumn. The timing of reproductive seasonality in many small ruminant species is controlled by the shortening of

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photoperiod (Chemineau et al., 1992) i.e., while the long photoperiods inhibit, the short photoperiods stimulate the reproductive activity (Bittman and Karsch 1984; Boden and Kennaway, 2006). Melatonin shows a low blood-concentration during daylight and a high concentration during darkness, thus it can be considered as an organic informer of the annual photoperiodic trend (Carcangiu et al., 2005; Carcangiu et al., 2013). Melatonin subcutaneous implants are used in Europe and other parts of the world, to advance reproductive activity of sheep in the spring (Abecia et al., 2006; Papachristoforou et al., 2007; deNicolo et al., 2008; Carcangiu et al., 2012). At high latitudes, the administration of melatonin by slow-release implants is usually performed around the summer solstice (Haresign et al., 1990), whereas, in the Mediterranean areas, around the spring equinox (Chemineau et al., 1996; Forcada et al., 1999). Several experiments have been carried out to determine which is the best time for treatment with melatonin, but the responses vary with breed (Staples et al., 1992; Abecia et al., 2007). The Sarda sheep (more than 3.0 million ewes farmed in Sardinia) is the main dairy breed in Italy and its production is parallels the pasture growth pattern (Carta et al., 2009). It exhibits a short anoestrous period, generally from early February until late March (personal observation). Administration of melatonin implants has been reported to improved fertility and advance lambing (Carcangiu et al., 2012; Luridiana et al., 2015). However, even in Sarda sheep the reproductive responses after treatment with melatonin are sometimes unsatisfactory, probably due to treatment occurring during the period of refractoriness (Abecia et al., 2007; Sweeney et al., 1997). This study aimed to evaluate the effect of melatonin treatment carried out between late winter and early spring on reproductive response in Sarda breed sheep and to determine whether photo-refractoriness influences this response.

## 2. Materials and methods

### 2.1. Experimental design

This study was conducted on 3200 Sarda breed ewes from 16 farms, located in North Sardinia (40°N). In order to minimize the farm effect, farms were selected according to comparable features such as total number of animals, milk production, flock health, veterinary recommendations (deworming, vaccinations and use of other pharmacological treatments), nutrition and reproductive management. All farms were located in the same area, within a 50 km radius of Sassari. During the day the animals grazed on legumes and grasses and they also received 300 g of commercial concentrate feed per head (crude protein 20.4% and 12.5 MJ ME/kg DM) during milking. The sheep were penned during night, and received hay (crude protein 11.1% and 7.2 MJ ME/kg DM) and water *ad libitum*. Each farm reared approximately 400 sheep, and 200 clinically healthy ewes were chosen from each farm for the study, accounting for a total of 3200 head. Ewes enrolled in the study were lactating, 3–6 years old, with body condition score (BCS) 2.5–4.0 and with a single lamb born between 1st November and 10th December 2013. The individual rumen bolus number was recorded, and each ewe was individually marked with a numbered collar to avoid mistakes in the animals' identification.

### 2.2. Treatment and registration data

On each farm the enrolled animals were randomly subdivided into 2 groups, each containing 100 ewes. The ewes in group M received one melatonin implant (18 mg) (Melovine, CEVA Sanità Animale, Agrate Brianza, Milano) in the left retro-auricular area; the other group of ewes served as control and remained untreated (group C). In the first 4 randomly selected farms melatonin implants were administered on 10th February, in the second 4 on 10th March, in the third 4 on 10th April and in the last 4 on 10th May; controls and treated animals were kept together all the time. Adult rams (male/female ratio 1/20) were introduced on each farm 35 days after treatment of ewes and remained in the flock for 45 days. Before ram introduction, ewes were separated from rams for at least 3 months. Rams received 3 melatonin implants, in the left retro-auricular area, 1 week before ewes. Gestation was diagnosed starting from 45 days and up to 90 days after the rams introduction by transabdominal ultrasonography examination using Esaote Piemedical Tringa linear equipment (Esaote Europe B.V., Maastricht, The Netherlands) provided with a 5.0–7.5 MHz multiple frequency linear probe. For each ewe the lambing date and number of new-born lambs were recorded from 150th to 190th day after ram introduction.

### 2.3. Statistical analysis

All statistical analysis was performed using the computing environment R, version 3.3.2. A logit-link Hierarchical Linear Model (HLM) appropriate for binomial (lambing/not lambing) data was used to analyse the fertility of different treatment time. Variables considered were treatment and treatment time. To analyse the effect of treatment time on period in days from male introduction to lambing a HLM procedure according to the following model was performed.

$$Y_{ijk} = \mu + Tm(Pe)_{ij} + \varepsilon_{ijk}$$

where Yijk is the period in days from male introduction to lambing, μ is the overall mean, Tm is the fixed effect of treatment, Pe is the nested time effect within treatment, and $\varepsilon_{ijk}$ is the error effect. The same model was used to analyse the litter size. A P value $< 0.05$ was considered statistically significant. Multiple comparisons of the means were performed using Tukey's method.