



# Melatonin treatment in spring and reproductive recovery in sheep with different body condition score and age



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## ABSTRACT

With the aim to evaluate the effect of melatonin treatment on the advanced reproductive recovery in sheep with different body condition score (BCS) and age, 800 ewes were selected from two farms. These ewes (3–6 years old, multiparous and with BCS 2.5–4.0) were subdivided into two Groups (200 M and 200 C), balanced on their BCS and age. On 20 March, Group M was treated with one melatonin implant (18 mg). Group C was untreated. Males were introduced to the groups 35 days after treatment. Gestation was diagnosed between day 45 and 90 after mating by transabdominal ultrasonography. From day 150 to 190 after rams introduction, lambing date and newborns' number were recorded. The average time in days between male introduction and lambing resulted shorter in treated than in control ewes ( $166.4 \pm 0.48$  vs.  $172.5 \pm 0.50$ ) ( $P < 0.05$ ). At day 160 and 170 from ram introduction the fertility rate was higher in Group M than in C ( $P < 0.05$ ). The overall fertility at day 190 from rams introduction showed no differences between Group M and C (337 and 339, respectively). At day 170 from male introduction the number of the 5–6 years-old lambed ewes were 2-fold higher than the youngers ( $P < 0.05$ ). The animals with a BCS 3.5–4.0 had a faster response to male effect, and a shorter mean distance in days from rams introduction to lambing, compared to those scored 2.5–3.0 ( $166.1 \pm 0.48$  vs.  $174.8 \pm 0.51$ ) ( $P < 0.05$ ). We concluded that the ewes with BCS 3.5–4.0 and aged 5–6 years showed a better response to melatonin treatment in spring.

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## 1. Introduction

The productivity in many breeds of sheep is limited by reproductive seasonality, which is under the influence of daylength (Yeates, 1949; Karsch et al., 1984; Ortavant et al., 1985). These photoperiodic effects on reproduction are mediated by the secretion of melatonin from the pineal gland (Bittman et al., 1983; Bittman and Karsch, 1984). Since melatonin is secreted during the night, decreasing day length is related with a longer pattern of melatonin

secretion, which is stimulatory to reproduction in short-day breeders, like sheep (Karsch et al., 1984; Robinson et al., 1985). Exogenously administered melatonin from continuous slow-release implants has been shown to advance the onset of the breeding season in sheep by mimicking the stimulatory effect of short days (Haresign et al., 1990). The insertion of microimplants containing melatonin in the vicinity of some hypothalamic nuclei stimulates the secretion of luteinizing hormone (LH) (Malpaux et al., 2001). The administration of melatonin, with different modalities, has shown to improve the reproductive efficiency in different sheep breeds, and subcutaneous implants are the most widely used both in Europe and in other continents (Chemineau et al., 1996; de Nicolo et al., 2009). The

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melatonin implants are able to mimic the short days, without causing an inhibition of pineal secretion of indoleamine, and stimulate reproductive activity in sheep (Staples et al., 1992). In dairy sheep, at Mediterranean latitudes, the advance of lambing at the beginning of Autumn is an essential condition for a duration of 6–7 months of lactation (Carcangiu et al., 2012). Thus, in order to allow lambings in Autumn, sheep should reproduce in early spring, during their anoestrus period. Moreover, in Autumn dairy sheep, such as the Sarda breed, are lactating and then it determines that the energies are used primarily for the milk production (Carcangiu et al., 2012). This fact could lead to having animals with a different body condition linked to their production level. 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 indoleamine could be masked by other factors such as age and nutritional state. However, melatonin treatment improved reproductive efficiency better in animals with a low BCS (2.5–3.0) than in those with a high BCS (>3.5) (Forcada et al., 1995); whereas, in untreated sheep a high BCS reduces the period of anoestrus compared to a low BCS (Forcada and Becia, 2006). Additionally, the reproductive response to the melatonin treatment is also influenced by age, in fact in sheep over 10 years, the administration of melatonin restores the pituitary sensitivity to GnRH (Forcada et al., 2007). 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 BCS and age.

## 2. Material and methods

### 2.1. Animal management and experimental design

For the present research two farms located in Sardinia between the 39° and 40°N has been identified. Each farm raised approximately 800 heads and showed similar structural facilities and managerial conditions. 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. The sheep were penned at night, and received hay (crude protein 11.1% and 7.2 MJ ME/kg DM) and water *ad libitum*. On 15th March 2012, in the first farm we identified 400 lactating ewes, aged between 3 and 6 years which lambled between 1st November and 10th December 2011. The number of the ear tag of each animal was recorded, and the ewes were individually marked with numbered collars to avoid recognition errors. At the same time the age and the date of the previous lambing were recorded. The day after (on 16th March 2012) the above procedure was performed in the second farm. In each farm the 400 selected sheep were distributed as follows: 200 with BCS 2.5–3.0, of which 100 aged 3–4 years and 100 aged 5–6 years, and the other 200 with BCS 3.5–4.0, of which 100 aged 3–4 years and 100 aged 5–6 years. Keeping the same proportion between BCS and age, in each farm the 400 animals were subdivided into two groups: 200 in Group M and

200 in Group C. Ewes at their first (ewe lambs) and second lambing were excluded to avoid influences related to their lambing periods. Indeed, the lambing periods of primiparous Sarda ewes generally occur between January and April, and the peak of lambing occurs between February and March. Because the Sarda breed exhibits high milk production levels, this breed clearly has poor reproductive efficiency during the two months following lambing. This delay leads to an additional late lambing in the second year, that influence reproductive resumption. In this research, only animals that lambled within the first 10 days of December 2011 were selected, and neither the young ewes at first lambing nor those 2 years old (because they lambled after the first 10 days of December 2011) were included. For these reasons, the selected ewes were at least 3 years old so that they were at least at third lambing. The BCS were registered when the animals were chosen according to the methods of 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. The sheep with a BCS lower than 2.5 or higher than 4.0 were excluded from the study, because they are in a very few number in commercial flocks, as they are less productive and thus uneconomic.

On 20th March 2012 the animals of Group M ( $n=200$ ) were treated with a slow release implant containing 18 mg melatonin, in the left retroauricular region. Two hundred animals of Group C were reserved as control and received nothing to simulate conditions in commercial sheep flocks. Controls and melatonin-treated sheep were grazed together.

The ewes were isolated from rams for at least 6 months. Ram were kept far from ewes where females could not see or smell them. On 25th April 2012 rams were introduced to the flock to induce a ram effect, 1:20 male to female ratio, and removed after 40 days. Moreover, each ram was provided of marking harnesses for oestrous detection. The numbers of ewes with ram keel marks were registered each day. The keel colour was changed every 10 days. Gestation was diagnosed between 45th and 90th day after mating 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. From 22nd September 2012 to 5th November 2012 the lambing date and numbers of newborn lambs were recorded.

### 2.2. Statistical analysis

Analysis of variance (R statistical software, R-pakage 3.0.0, Lenth, 2013) was performed to analyse the association between melatonin treatment, BCS, age and reproductive activity (measured as the distance in days from ram introduction to lambing). The following linear model was used:

$$Y_{ijklmn} = \mu + F_j + G_k + N_m + D_l + G_k N_m + G_k D_l + N_m D_l + G_k N_m D_l + E_{ijklmn}$$

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