Oestrous activity, ovulation rate and plasma melatonin concentrations in Rasa Aragonesa ewes maintained at two different and constant body condition score levels and implanted or reimplanted with melatonin

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Abstract

Forty-seven adult and non-pregnant Rasa Aragonesa ewes were used to study the effect of two constant body condition score levels on reproductive parameters in ewes implanted in early April or reimplanted 49 days later with melatonin. The duration of the experiment was 9 months (27 November 1992–3 September 1993), and was designed as a factorial study defined by two constant body condition (BC) scores, high (H; 2.75 or greater) or low (L; 2.50 or less) and the implant on 8 April (M) or reimplant (2M) with melatonin (18 mg, Melovine™). Oestrus was detected daily using vasectomized rams and ovulation rate was measured by laparoscopy 6 days after positive identification. Blood samples were collected twice weekly from each ewe and assayed for progesterone. In addition, samples were collected from 20 ewes (five in each treatment group) every 2 h and hourly for 24 h on Days 27 and 84 after first implantation, respectively, and assayed for melatonin.

Onset of seasonal anoestrus was significantly influenced by the BC score (February 16 ± 8 and March 15 ± 5 for H and L groups; P < 0.01), but neither of the factors considered in the study influenced the interval between the first implantation (8 April) and the first detected oestrus (54 ± 7 days, 63 ± 8 days, 64 ± 10 days and 66 ± 5 days for HM, LM, H2M and L2M groups, respectively). Ovulation rate in the third cycle detected from 1 month after first implant insertion

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was significantly influenced by the BC score level (1.78 vs. 1.44 corpora lutea for H and L groups; \( P < 0.05 \)). However, ovulation rate in the first and second cycles was slightly lower in the H group (1.46 vs. 1.64 and 1.48 vs. 1.56 corpora lutea, respectively), suggesting a positive effect in the short term of the melatonin implants in ewes with a moderately low BC score. No significant effect of the reimplant on ovulation rate was detected. Plasma melatonin concentrations on Day 84 were not different between groups of ewes receiving one or two implants. Furthermore, and except for nocturnal levels in the high BC score group, ewes that received one implant showed significantly higher melatonin concentrations on Day 84 than on Day 27 after implantation. It is concluded that (i) interval between melatonin implantation and first oestrus is not influenced by the BC score level; (ii) the reimplant does not improve reproductive parameters, although exogenous melatonin seems to overcome the positive effect of a high BC score in the early breeding season; (iii) one implant is adequate to maintain high plasma melatonin levels up to 3 months after implantation.

**Keywords:** Sheep; Body condition; Melatonin; Sexual activity

### 1. Introduction

Photoperiod plays a critical role in regulating the seasonality of reproduction in sheep (Yeates, 1949), and the seasonal reproductive activity is generated by an endogenous circannual rhythm that is not driven by photoperiod but entrained by it (Malpaux et al., 1989). Photoperiodic information is conveyed to the reproductive neuroendocrine system by a circadian secretion of melatonin from the pineal gland (Bittman et al., 1983). Melatonin implants inserted around the summer solstice can be used to advance the breeding season in seasonal breeds (McMillan and Sealey, 1989; Haresign et al., 1990; Durotoye et al., 1991). However, it is important to establish the limitations of melatonin treatment as a practical means of manipulating reproductive activity in the ewe. Although long-term exposure to melatonin treatment of ewes is detrimental to the ovulation rate (Jordan et al., 1990), the effects of medium-term continuous exposure to melatonin are not defined.

Nutrition has an important impact on reproductive parameters in sheep, and an increase of ovulation rate has been associated with a higher body condition (BC) score (Xu et al., 1989; Rhind et al., 1989), especially in Mediterranean breeds at the beginning of the breeding season (Forcada et al., 1992). With regard to the use of melatonin in these genotypes to improve reproductive parameters after spring lambing when an accelerated breeding system (three lambings in 2 years) is used, Forcada et al. (1995) showed that the use of exogenous melatonin in April (32 days after lambing) may be an effective way of advancing the oestrous activity resumption in Salz ewes; ovulation rate can be also increased, especially associated with a low rather than a high plane of nutrition. However, the effect of a constant body fat reserve level on reproductive parameters of melatonin treated ewes in the early breeding season remains unknown.

Rasa Aragonesa breed is a local Spanish genotype that shows a short anoestrous period between May and July, although a variable percentage of ewes (20–40%) may exhibit oestrus every month of the year (Forcada et al., 1992). A higher interval between weaning and first behavioural oestrus has been observed after lambing in March (113
days; Abecia et al., 1993a) than during the first fortnight of June (60 days; Abecia et al., 1993b). Oestrous activity of this breed can be stimulated by a high BC score or plane of feed intake, although the most important effect of nutrition is performed on the reactivation of ovulation rate at the beginning of the breeding season (Forcada et al., 1992; Abecia et al., 1993a; Abecia et al., 1993b).

This study was designed to verify whether the effect of melatonin treatment on sexual activity and ovulation rate can be modulated by BC score in Spanish Rasa Aragonesa ewes implanted in early April or reimplanted 49 days later with melatonin.

2. Materials and methods

The study was conducted at the experimental farm of the University of Zaragoza, Spain (latitude 41° 40′ N), which meets the requirements of the Scientific Procedure and Breeding of Animals for use in Scientific Procedure Establishments, and holds the certificate of designation in the European Union No. 47186-17 AB. Forty-seven adult and non-pregnant Rasa Aragonesa ewes, which had lambed at least 5 months previously, were used. Animals were kept permanently in communal yards with an uncovered area throughout the experiment and without any supplementary light. Fresh water was available at all times.

The experiment started on 27 November 1992. The animals were divided into two groups on the basis of BC score (Russel et al., 1969): high (BC ≥ 2.75; H; n = 24) or low (BC ≤ 2.50; L; n = 23), and fed in groups (separately according to their BC score) with an allowance of 1 kg ammonia-treated straw, 0.1 kg soya meal and 2 kg (H) or 1 kg (L) pomace silage per head per day. These amounts correspond to a daily intake of 2.88 Mcal and 2.38 Mcal metabolisable energy (ME), supporting 1.5 and 1.25 times the maintenance requirements, respectively, for a standard ewe of 50 kg liveweight (ARC, 1980).

The duration of the experiment was 9 months (27 November 1992–3 September 1993). Ewes received on 8 April a single, subcutaneous implant at the base of the left ear containing 18 mg melatonin (Melovine™, CAMCO, Cambridge Animal and Public Health, Cambridge, UK and SANOFI, Santé Nutrition Animale, Libourne, France), and 49 days later (27 May) half of the ewes were reimplanted. Thus, both groups H and L were divided into subgroups of single melatonin implanted ewes (M; n = 23) and reimplanted ewes (2M; n = 24). From then on, the experiment was a 2 × 2 factorial study with BC score level and implant or reimplant as factors of the model. The experiment was divided into 16 periods each of 17 days according to the length of the sexual cycle in sheep. Oestrus was tested daily using aproned rams and ovulation rate was measured (except during the seasonal anoestrous period) by laparoscopy 6 days after positive identification of oestrus. Liveweight and BC score were determined once a week. In order to determine the percentage of silent ovoluations before the first behavioural oestrus, blood samples were collected twice weekly from each animal by jugular venipuncture and assayed for progesterone. In addition, samples were collected every 2 h and hourly for 24 h on Days 27 and 84 after first implant insertion, respectively, from 20 ewes (five in each treatment group) via a jugular catheter,
Plasma progesterone concentrations were analysed by radioimmunoassay in duplicate (50 μl of plasma) using a direct solid-phase ¹²⁵I assay (Carlson et al., 1989) with rabbit antiserum (bioMérieux sa, Marcy L’Etoile, France). Sensitivity of the assay was 0.05 ng ml⁻¹, and intra and inter-assay coefficients of variation were 11.6% and 13.7%, respectively. Plasma melatonin levels were analysed by radioimmunoassay in duplicate (100 μl of plasma) in a single assay using the technique of Fraser et al. (1983) with an antibody first raised by Tillet et al. (1986). The sensitivity of the assay was 4 pg ml⁻¹ (melatonin Fluka-63610), with an intra-assay coefficient of variation of 12%.

Criteria used to evaluate oestrous and ovarian activities were: (i) percentage of ewes showing oestrus during each 17-day period; (ii) end of breeding season (date of the last detected oestrus) and length of seasonal anoestrus (period between final oestrus and the first of the next breeding season); (iii) interval between melatonin implant and first detected oestrus; (iv) ovulation rate of each detected cycle (especially from 1 month after first implant insertion); (v) percentage of ovulations unaccompanied by oestrus (silent ovulations); the minimum plasma progesterone level to indicate a luteal phase was 0.5 ng ml⁻¹, which is the minimum value for monitoring cyclicity used previously in Rasa Aragonesa ewes (Folch et al., 1985; Forcada et al., 1992) and its crosses (Forcada et al., 1991).

For each 24-h set of samples, melatonin night levels were defined according to Malpaux et al. (1987). A melatonin elevation was defined as the interval between the first and the last value that exceeded the baseline level preceding and following the dark period by more than three standard deviations of those respective baselines (the baselines were defined as the means of the daytime samples preceding and following the dark period).

The incidence of ovarian activity and detected oestrus between groups was compared using a χ² test. Means (± SEM) were calculated for liveweight and BC score, end of breeding season, length of seasonal anoestrus, implant–first oestrus interval, ovulation rate and plasma melatonin levels. A 2 × 2 factorial analysis of variance was used to evaluate effects of BC score level and implant or reimplant of exogenous melatonin on length of seasonal anoestrus, onset of oestrous activity and ovulation rate after first implantation, and plasma melatonin levels at Day 84 after first implant.

3. Results

Mean liveweight and BC score of ewes at the beginning and the end of the experiment are shown in Table 1. Although no differences within groups were observed in BC score throughout the studied period, final liveweight was slightly higher than the initial one. Differences in liveweight and BC score between high and low body fat reserve groups were significant (P < 0.01 and P < 0.001, respectively). Average levels of both parameters can be considered as moderately high or low associated with normal nutritional conditions frequently encountered in commercial flocks. No significant effect
Liveweight (LW) and body condition (BC) score at the beginning and the end of the experiment in Rasa Aragonesa ewes with a moderately high (H) and low (L) body condition score. Means ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>H (n = 24)</th>
<th>L (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial LW (kg)</td>
<td>53.0±1.0 a</td>
<td>47.9±1.1 b</td>
</tr>
<tr>
<td>Final LW (kg)</td>
<td>55.5±1.2 a</td>
<td>50.0±1.5 b</td>
</tr>
<tr>
<td>Initial BC</td>
<td>2.92±0.05 a</td>
<td>2.50±0.13 b</td>
</tr>
<tr>
<td>Final BC</td>
<td>2.91±0.11 a</td>
<td>2.48±0.11 b</td>
</tr>
</tbody>
</table>

Different superscripts in the same row indicate significant differences of at least P < 0.01.

Liveweight (LW) and body condition (BC) score at the end of the experiment in H and L groups subdivided into animals implanted (M) or reimplanted (2M) with exogenous melatonin. Means ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>HM (n = 12)</th>
<th>LM (n = 11)</th>
<th>H2M (n = 12)</th>
<th>L2M (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final LW (kg)</td>
<td>55.4±1.9</td>
<td>51.0±2.4</td>
<td>55.7±1.5</td>
<td>49.0±1.8</td>
</tr>
<tr>
<td>Final BC</td>
<td>2.98±0.16</td>
<td>2.48±0.17</td>
<td>2.83±0.15</td>
<td>2.48±0.16</td>
</tr>
</tbody>
</table>

Onset of seasonal anoestrus (date of last detected oestrus) was significantly influenced by the BC score level (Day 47 ± 8 (February 16) vs. Day 74 ± 5 (March 15) for H and L groups, respectively; P < 0.01). Seasonal anoestrus period was 57 days shorter in the H group (P < 0.001) (Table 3), with no significant effect of the implant or reimplant with exogenous melatonin. Neither of the factors considered influenced the end of seasonal anoestrus or the first implant (April 8) to first detected oestrus interval (Table 3).

Sexual activity (percentage of detected oestrus) in each 17-day period was higher in the H group before and during the seasonal anoestrous period (Fig. 1). Differences were significant (P < 0.05) from period 4 (second fortnight of January) to period 10 (first fortnight of May), although they were greater in periods 6 and 7 (end of February and March) (92% and 71% vs. 43% and 26% respectively for H and L groups; P < 0.01), just before the beginning of the seasonal anoestrus. Level of fat reserves or reimplant with exogenous melatonin did not influence percentage of oestrous activity after the onset of the new breeding season.

Percentage of ovarian cyclicity indicated by plasma progesterone concentrations matched levels of sexual activity assessed by oestrous detection with rams, revealing a small number of ovulations unaccompanied by oestrus, with an average percentage throughout the experiment of 3% and 9% for H and L groups.

Ovulation rate before the onset of seasonal anoestrous period was not different between BC score groups, although the ovulatory potential of H ewes was slightly higher (Fig. 1). There was a significant effect of the BC score level in the third sexual
Table 3

Length and end of seasonal anoestrus, and interval between first implant and first detected oestrus in Rasa Aragonesa ewes with a high (H) or low (L) body condition (BC) score and implanted (M) or reimplanted (2M) with exogenous melatonin (ME). Means ± SEM

<table>
<thead>
<tr>
<th></th>
<th>HM</th>
<th>LM</th>
<th>H2M</th>
<th>L2M</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of seasonal anoestrus (days)</td>
<td>61 ± 11</td>
<td>113 ± 18</td>
<td>70 ± 17</td>
<td>130 ± 14</td>
<td>+ ** NS</td>
</tr>
<tr>
<td>End of seasonal anoestrus (date)</td>
<td>Jun 1 ± 7</td>
<td>Jun 10 ± 8</td>
<td>Jun 11 ± 10</td>
<td>Jun 13 ± 5</td>
<td>NS NS</td>
</tr>
<tr>
<td>Interval implant–first oestrus (days)</td>
<td>54 ± 7</td>
<td>63 ± 8</td>
<td>64 ± 10</td>
<td>66 ± 5</td>
<td>NS NS</td>
</tr>
</tbody>
</table>

Values in parentheses are the number of animals.

* Five animals were cyclic throughout the experiment.

NS, not significant; + * P < 0.01.

cycle of the new breeding season (1.78 vs. 1.44 corpora lutea for H and L groups, respectively; P < 0.05). However, ovulation rate in the first and second cycles was slightly lower in the moderately high body fat reserve group (1.46 vs. 1.64 and 1.48 vs. 1.56 corpora lutea, respectively). No significant influence of the reimplant on ovulatory potential was detected.

Fig. 1. Oestrous activity (OA) (%) and ovulation rate (OR) throughout the studied period in Rasa Aragonesa ewes with a moderately high (H) or low (L) body condition score. * P < 0.05 when compared with L; ** * P < 0.01 when compared with L.
The 24 h patterns of mean plasma melatonin concentrations during each collection period (Days 27 and 84 after first implant insertion) in relation to the level of nutrition are illustrated in Figs. 2 and 3, respectively. Plasma melatonin levels during both intensive samplings showed a clear night/day rhythm in implanted or reimplanted animals, with significantly higher concentrations at night (at least $P < 0.05$), with no significant effect of the BC score group. Mean night and daytime plasma melatonin concentrations between ewes with one or two implants on Day 84 after first implantation (35 days after reimplant) were not significantly different. Ewes that received only one implant showed a significantly higher melatonin level (at least $P < 0.05$) during the diurnal and nocturnal periods on Day 84 than on Day 27 after implantation except for night time levels in the high BC score group (Table 4).

4. Discussion

A moderately high BC score was associated in this study with a shorter seasonal anoestrous period, this effect being more pronounced at the end of the breeding season than at the end of the seasonal anoestrus, as the latter seems to be less influenced by nutrition (Montgomery et al., 1988; Forcada et al., 1992; Abecia et al., 1993b).

Mean interval between first implant insertion and first behavioural oestrus was not influenced by the BC score level (64 ± 4.7 days and 59 ± 6.1 days for L and H groups, respectively). This interval was similar to that reported at the same latitude by Forcada et al. (1995) in Salz ewes (50% Romanov and 50% Rasa Aragonesa) (Sierra, 1982) lambing in the second fortnight of March and implanted with exogenous melatonin on
Fig. 3. Twenty-four hour plasma melatonin profiles in implanted (•) or reimplanted (+) Rasa Aragonesa ewes with a moderately high (a) or low (b) body condition score on Day 84 (1 July) after first implant insertion. Means±SEM for n = 5 except for HM group (n = 4).
Table 4

Night and daytime plasma melatonin concentrations on Days 27 and 84 after implantation in Rasa Aragonesa ewes receiving only one implant and with a moderately high (HM) and low (LM) body condition score. Means ± SEM

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>HM</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diurnal period (pg ml⁻¹)</td>
<td>27</td>
<td>191±39</td>
<td>231±25</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>324±48*</td>
<td>394±37 **</td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal period (pg ml⁻¹)</td>
<td>27</td>
<td>347±70</td>
<td>349±49</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>484±69</td>
<td>636±72 **</td>
</tr>
<tr>
<td></td>
<td>(n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05 when compared with Day 27; ** P < 0.01 when compared with Day 27.

Day 32 after lambing (51 days). Other authors have reported a mean interval between the start of the melatonin treatment and the onset of ovarian activity of 59 days in implanted animals at the beginning of May (Chemineau et al., 1993) and 76 days after daily oral administration starting on 5 March at 57°N (Robinson et al., 1992). It has been shown that melatonin implantation in sheep conveys a short-day photoperiod signal in prolonging the breeding season (O’Callaghan et al., 1991), but the way in which melatonin implants act to advance sexual activity, either as a short day or rendering ewes nonphotoperiodic, is not clear. In fact, the effectiveness of exogenous melatonin depends on the time of the start of treatment (later as latitude increases) (Robinson et al., 1997; Staples et al., 1992) and the genotype (I. López and Inskeep, 1991; Haresign, 1992). Thus, genetic differences in photoperiodic regulation of the reproductive rhythm have been detected (O’Callaghan et al., 1992). Likewise, a high individual variation in sensitivity to melatonin implants has been also observed (Chemineau et al., 1993; Forcada et al., 1995).

The number of ovulations unaccompanied by oestrus recorded in the present study was less than 10%. These results are opposed to those reported previously in the same genotype (Forcada et al., 1992) or in other Mediterranean breeds (Thimonier and Mauléon, 1969; Avdi et al., 1988), where it is revealed the existence of an elevated number of silent ovulations, especially in the seasonal anoestrous period.

An increase of ovulation rate in the breeding season has been associated with a high BC score (Rhind et al., 1989; Xu et al., 1989; Forcada et al., 1992). However, the similar ovulation rate in the first and second cycles detected from 1 month after first implant insertion found in the present study in both of the body fat reserve groups suggests that the short-term effect of the melatonin implants on this reproductive parameter seems to be greater in ewes with a moderately low BC score. It is possible that the effect of fat reserves on ovulation rate in implanted animals was performed later in the third sexual cycle. Other authors have found a significant interaction between plane of nutrition and exogenous melatonin on ovulatory ability in the short term, showing that the effect of melatonin treatment around the spring equinox in enhancing
ovulation rate is more pronounced in ewes on a low compared with a high plane of feed intake (Robinson et al., 1991; Forcada et al., 1995).

No effect of the reimplant on ovulation rate was detected. This would indicate that high plasma melatonin levels induced for a long time by treatment could counteract the positive influence of a moderately high level of body fat reserves. Jordan et al. (1990) reported that prolonged exposure to exogenous melatonin has a detrimental effect on ovulation rate in the long term.

Analysis of plasma melatonin concentrations showed that the difference between night and daytime levels was similar in the different groups studied for both sampling tests in spite of the higher concentrations showed on Day 84. This shows that exogenous melatonin does not seem to exert any negative feedback on the endogenous secretion of the hormone by the pineal gland (Lincoln and Ebling, 1985).

A higher amplitude of plasma melatonin concentrations at the second sampling test (1 July), with a shorter night period, was detected for ewes that received only one implant. This photoperiod-associated tendency confirms the results previously obtained in reduced seasonality ewes implanted in April. Likewise, Arendt et al. (1981) reported a lower amplitude profile of melatonin in winter than in summer. Furthermore, the lack of differences in plasma melatonin concentrations between implanted or reimplanted ewes on Day 84 after first implantation shows that, under the present conditions, the use of the reimplant is not required, as the Rasa Aragonesa ewes are able to maintain high plasma melatonin levels up to 3 months after the beginning of the treatment.

In conclusion, the results of this study show that BC score level does not alter the interval between implantation with melatonin and first behavioural oestrus. Melatonin treatment could be useful to improve ovulation rate in ewes with a moderately low body fat reserve level at the beginning of the breeding season. Moreover, the reimplant does not improve reproductive parameters, and Rasa Aragonesa ewes maintain high plasma melatonin levels with the use of a single implant up to 3 months after the start of the treatment.

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References


