The effect of melatonin on the reproductive performance of three sheep breeds in Spain

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Abstract

In order to determine the efficacy of melatonin implants in initiating the lambing season and improving reproductive efficiency in sheep, ewes (n = 1932) from three breeds (Rasa Aragonesa, Assaf and Merino) received melatonin implants (Group M) every 45 days from the winter solstice (1 January and 15 February—winter; 1 April and 15 May—spring) on 12 commercial farms. The other group of ewes (n = 1715) served as control (Group C). Fertility (percentage of ewes lambed), litter size (number of lambs born per ewe lambed), and fecundity (number of lambs born per ewe in the group) were calculated. Rasa Aragonesa M ewes produced significantly more lambs than the C ewes on farms 3 and 4, after spring melatonin treatment: April (1.16 versus 0.83 lambs-fecundity; P < 0.01) and May (1.23 versus 0.97 lambs; P < 0.05) lambings, respectively. In the Assaf ewes, melatonin implants significantly improved fecundity on farms 5, 6 (winter): January (0.62 versus 0.42 lambs per ewe for M and C groups; P < 0.01) and February (0.64 versus 0.32 lambs; P < 0.01) lambings, respectively and on farm 8 (spring) for May (0.77 versus 0.54 lambs; P < 0.01). This improvement in fecundity is attributed exclusively to significantly higher fertility during these melatonin implantation periods. Concerning the Merino breed, the melatonin treatment increased fecundity only on farm 10, after winter treatment (February) (0.91 versus 0.61 lambs for M and C groups; P < 0.01). It can be concluded that melatonin could be a useful tool to improve lamb production in the three breeds under study, although its efficacy within each breed seems to vary according to the farm and season.

Keywords: Sheep; Melatonin; Fertility; Litter size; Season

1. Introduction

Productivity in farm species, especially sheep, is limited by seasonality, which in turn is regulated by photoperiod (Yeates, 1949). Information regarding photoperiod is conveyed to the neuro-endocrine system by the circadian secretion of melatonin from the pineal gland (Bittman et al., 1983). Thus, subcutaneous implants of this hormone were designed as a means of inducing artificial control of oestrous activity in sheep. Melatonin is naturally released at night, but subcutaneous implants can be used to increase the concentration over 24 h and cause a short-day-like response, without suppressing the endogenous secretion (O’Callaghan et al., 1991; Malpaux et al., 1997). The commercial use of exogenous melatonin in sheep has been authorised in several countries of the European Union.

In adult ewes, melatonin implants have been traditionally inserted around the time of the summer solstice in order to advance the breeding season (McMillan and Sealey, 1989; Haresign et al., 1990). Unlike the Northern European sheep breeds, the Mediterranean breeds have
a short seasonal anoestrus, especially when social (ram effect) or nutritional factors are managed appropriately (Lindsay, 1996). Mediterranean sheep breeds show an earlier onset of the breeding season, compared to genotypes located at higher latitudes, even when both are subjected to the same photoperiodic treatment (Martin et al., 1999). Therefore, melatonin implants in Mediterranean flocks at commercial level are usually inserted at approximately about the time of the spring equinox (Chemineau et al., 1996; Forcada et al., 1999).

Research has demonstrated the efficacy of melatonin implants when inserted immediately after the winter solstice in the Rasa Aragonesa sheep breed, either advancing the reproductive activity in oestradiol-treated, ovarioctomized ewes in the absence of males, or improving reproductive parameters under field conditions during the subsequent mating period (Forcada et al., 2002). These results have been confirmed in oestradiol-treated, ovarioctomized ewes implanted every 45 days from 1 January (Valares et al., 2004). Thus, it has been hypothesised that implantation with melatonin from the winter to the summer solstice could open new possibilities in the development of breeding programmes in ewes with a short seasonal anoestrous period.

After 5 years of commercializing melatonin implants in Spain, the objective of this work was to determine the efficacy of melatonin implants at the commercial level by inserting the implants every 45 days throughout the period of increasing photoperiod (winter and spring), to improve the reproductive performances of three breeds of sheep reared in Spain, namely the Rasa Aragonesa, Assaf and Merino.

2. Materials and methods

This study was conducted on 12 commercial Spanish farms, located at Zaragoza (41°N) (Rasa Aragonesa), Zamora (42°N) (Assaf) and Badajoz (38°N) (Merino). The Rasa Aragonesa breed (more than 2.5 million ewes) has a short anoestrous period (<100 days) in spring, although a variable percentage of females (20–50%) show ovarian activity throughout the year (Forcada et al., 1992). The Spanish Merino is the origin of the finest wool sheep breed in the world, and the most important breed in Spain as measured by the number of ewes (more than 3 million). This breed is mainly monovular, and has a high percentage of ewes ovulating even during the theoretical seasonal anoestrous period (50–70%) (González, 1993). Both breeds are well adapted for intensive production and accelerated lambing programmes. The Assaf is the main dairy sheep breed in Israel, created by crossbreeding of the East Friesian (5/8) with the Awassi (3/8) breed (Goot, 1986). It was exported to other Mediterranean countries, and currently, more than 1.5 million Assaf ewes are used in milk production in Spain. Although there are no available data in the literature regarding its sexual seasonality, Palacios et al. (2002) reported the Spanish Assaf ewes to be more seasonal than other breeds (Merino or Lacaune) reared in Spain at the same latitude.

The field trials were carried out in 12 farms, four for each breed, involving more than 3600 animals. The particular experimental conditions of this work and its objectives (three breeds located at three different latitudes, four different times of melatonin treatment and the high number of animals involved) compelled the authors to carry out the trials on commercial farms—one for each period and breed, so that farm and time of implantation effects are confounded. In an attempt to minimize the effect of farm on the results, relatively homogeneous farms were selected following criteria such as total number of animals in the farm, nearness (short distance between farms), source of animals, milk production, veterinary recommendations, nutrition, and especially reproductive management. The sheep were considered representative of the flocks for each breed and localization. The farms with the Rasa Aragonesa and Assaf breeds were located within a 200 km radius of the same area. The Rasa Aragonesa flocks belonged to the National Association of Rasa Aragonesa Breeders (ANGRA) and are controlled by the same veterinary team. The Assaf flocks were integrated in a cooperative (ASOVINO), which has implemented the same reproductive protocols on its farms. Merino flocks were located in the same village, and were advised by the same veterinary team. The Rasa Aragonesa and Merino flocks were managed in an accelerated breeding programme, with a lambing interval of 8 months and an average lactation period of 45 days. The dairy Assaf farms used recorded an average milk production of 1.71 l/d for a milking period of 6–7 months. Ewes received melatonin implants approximately 60 days post-partum.

A total of 3683 adult ewes were used in the trial. Experimental ewes were classified as “normal” considering their previous reproductive performances for 3 years—all of them having lambed the previous year. Melatonin implants were inserted from January to May, approximately every 45 days (1 January, 15 February, 1 April, and 15 May). A group of ewes were randomly chosen to receive a subcutaneous implant containing 18 mg of melatonin (Melovine, CEVA Salud Animal, Barcelona, Spain) (Group M). Similarly, another group of ewes were allotted as non-implanted, control ewes (Group C). The number of ewes of each breed and farm,
Table 1
Number of ewes in each farm and time of treatment with (M) or without receive (C) a subcutaneous melatonin implant

<table>
<thead>
<tr>
<th>Number of farms</th>
<th>Time of treatment</th>
<th>M</th>
<th>C</th>
<th>Rams introduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 January (winter)</td>
<td>67</td>
<td>74</td>
<td>12 February</td>
</tr>
<tr>
<td>2</td>
<td>12 February (winter)</td>
<td>255</td>
<td>185</td>
<td>26 March</td>
</tr>
<tr>
<td>3</td>
<td>10 April (spring)</td>
<td>199</td>
<td>202</td>
<td>22 May</td>
</tr>
<tr>
<td>4</td>
<td>10 May (spring)</td>
<td>194</td>
<td>198</td>
<td>21 June</td>
</tr>
<tr>
<td>Rasa Aragonesa</td>
<td></td>
<td>715</td>
<td>659</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10 January (winter)</td>
<td>212</td>
<td>113</td>
<td>21 February</td>
</tr>
<tr>
<td>6</td>
<td>8 February (winter)</td>
<td>122</td>
<td>99</td>
<td>22 March</td>
</tr>
<tr>
<td>7</td>
<td>14 April (spring)</td>
<td>130</td>
<td>135</td>
<td>2 June</td>
</tr>
<tr>
<td>8</td>
<td>9 May (spring)</td>
<td>208</td>
<td>200</td>
<td>19 June</td>
</tr>
<tr>
<td>Assaf</td>
<td></td>
<td>672</td>
<td>547</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4 January (winter)</td>
<td>100</td>
<td>100</td>
<td>15 February</td>
</tr>
<tr>
<td>10</td>
<td>15 February (winter)</td>
<td>199</td>
<td>201</td>
<td>29 March</td>
</tr>
<tr>
<td>11</td>
<td>2 April (spring)</td>
<td>200</td>
<td>199</td>
<td>14 May</td>
</tr>
<tr>
<td>12</td>
<td>6 May (spring)</td>
<td>46</td>
<td>45</td>
<td>24 Jun</td>
</tr>
<tr>
<td>Merino</td>
<td></td>
<td>545</td>
<td>545</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1932</td>
<td>1751</td>
<td></td>
</tr>
</tbody>
</table>

Farms 1–4, Rasa Aragonesa; Farms 5–8, Assaf; Farms 9–12, Merino.

The actual date of melatonin implantation and ram introduction is set out in Table 1. The procedure was similar on all 12 farms. Ewes were isolated from rams at least 2 months prior to melatonin implantation. Rams (1 ram:15 ewes) received three implants one week before the ewes were treated, and 6 weeks later were introduced into each group to induce a ram effect. Rams remained in the flock for 45 days.

The criteria used to evaluate the effect of the treatment were lambing rate or fertility (percentage of ewes lambed); litter size or prolificacy (number of lambs born per ewe lambed) and fecundity (number of lambs born per ewe of the group). In order to obtain an objective measurement of the reproductive performance, the number of additional lambs born per melatonin treated ewe and increment (%), were calculated.

Fertility, litter size and fecundity were recorded after parturition. Fertility rate was compared with the aid of a $\chi^2$-test. In order to elucidate a different response to melatonin treatment in relation to the implantation time within each breed, litter size and fecundity were compared using a factorial analysis of variance according to the following fixed effect model: $Y = Xb + e$; where $Y$ is the $N \times 1$ vector of records, $b$ denotes the fixed effects in the model (four dates-four farms and two treatments) with the associated matrix $X$, and $e$ denoted the vector for residual effects. As the interaction between fixed effects was not significant, it was omitted from the model, and the effect of melatonin on the reproductive parameters in each treatment time/farm was compared separately, using a one-way analysis of variance (SPSS, 2005).

3. Results

A significant effect of farm and time of melatonin treatment was recorded for fertility, litter size and fecundity in the three breeds under study (Rasa Aragonesa, $P < 0.0001$; Assaf, $P < 0.05$; Merino, $P < 0.0001$). Melatonin treatment increased the fertility rate significantly in the two spring treatment periods (1 April-Farm 3, $P < 0.01$, and 15 May-Farm 4, $P < 0.05$) in the Rasa Aragonesa flocks. Regarding litter size, the melatonin-treated ewes recorded a significant higher number of lambs born after treatment in January-Farm 1 (winter, $P < 0.05$) and April-Farm 3 (spring, $P < 0.01$). The melatonin implants improved the fecundity in the Rasa Aragonesa ewes when inserted in spring (1 April-Farm 3; $P < 0.01$ and 15 May-Farm 4; $P < 0.05$) in the Rasa Aragonesa flocks. Regarding litter size, the melatonin-treated ewes recorded a significant higher number of lambs born after treatment in January-Farm 1 (winter, $P < 0.05$) and April-Farm 3 (spring, $P < 0.01$). The melatonin implants improved the fecundity in the Rasa Aragonesa ewes when inserted in spring (1 April-Farm 3; $P < 0.01$ and 15 May-Farm 4; $P < 0.05$). The highest increment in the number of lambs born per ewes treated in this breed was obtained in April-Farm 3 (40%) (Figs. 1–3).
Fig. 1. Fertility, litter size and fecundity of Rasa Aragonesa ewes treated (■) with melatonin or not (□) (*P < 0.05; **P < 0.01).

(spring) (Farms 5, 6 and 8, respectively). The highest increase in the number of lambs born per ewe treated was obtained on Farm 6 (February) (131%). This percentage was 48 and 43 % for January-Farm 5 and May-Farm 8, respectively (Table 2).

Melatonin implants significantly improved fertility in Merino ewes after treatment in February-Farm 10 and April-Farm 11 (P<0.01). The prolificacy was only improved following melatonin treatment in ewes implanted during February-Farm 10 (P<0.01). This parameter was higher for the control ewes on Farm 11 (April) (P<0.01). The fecundity of the Merino ewes treated with melatonin was significantly higher only when implanted in February-Farm 10 (winter, P<0.01), recording an increase in the number of lambs born per treated ewe of 49% (Table 2).

Table 2
Number of extra lambs born per melatonin treated ewe and gain (%) for the three breeds and in the farms under study

<table>
<thead>
<tr>
<th>Farm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rasa Aragonesa</td>
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<tr>
<td>Extra lambs born/treated ewe</td>
<td>NS</td>
<td>NS</td>
<td>0.33</td>
<td>0.26</td>
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<td></td>
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<td></td>
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<tr>
<td>% Increment</td>
<td>NS</td>
<td>NS</td>
<td>40</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Assaf</td>
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<tr>
<td>Extra lambs born/treated ewe</td>
<td>0.20</td>
<td>0.42</td>
<td>NS</td>
<td>0.23</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Increment</td>
<td>48</td>
<td>131</td>
<td>NS</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Merino</td>
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<tr>
<td>Extra lambs born/treated ewe</td>
<td>NS</td>
<td>0.30</td>
<td>NS</td>
<td>NS</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Gain</td>
<td>NS</td>
<td>49</td>
<td>NS</td>
<td>NS</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Time of treatment: January (Farms 1, 5, 9), February (Farms 2, 6, 10), April (Farms 3, 7, 11) and May (Farms 4, 8, 12).
Results in the present study show exogenous melatonin treatment to improve the production of lambs in the different Mediterranean breeds of sheep. However, results are different when either breed or dates of treatment are considered. In the present experiment, and in spite of the attempt to equalize farming conditions, farm and date effects were confounded, and the results need to be carefully analysed and interpreted. Thus, in a trial designed to evaluate melatonin treatment on 21 commercial sheep farms in France, Chemineau et al. (1991) recorded significant farm and melatonin effects on the reproductive performance, but an absence of interaction between both parameters. Similarly, Haresign (1992), in an experiment involving 18 farms and two sheep breeds in the UK, found a considerable degree of variation between flocks in the number of extra lambs produced per ewe exposed to the ram. However, it was concluded that much of this could be accounted for by seasonal differences in the time of treatment. This could be the cause of the differences between farms observed in this experiment within breed, as ewes were treated from January (winter) to May (spring). In a third field trial carried out in Spain, it was concluded that the potential of Spanish sheep to respond to melatonin treatment varies in relation to the breed, month and reproductive history of the dam (López and Inskeep, 1991).

Melatonin treatment was effective in Rasa Aragonesa ewes after treatment in spring (April and May), on Farms 3 and 4, respectively, where 0.30 extra lambs per ewe treated were obtained. This improvement is very similar to that previously reported for the same breed treated on 22 March (spring), and is associated with a significant increase in both fertility and prolificacy (Forcada et al., 2002). According to Zúñiga et al. (2002), melatonin increases the number of ewes in oestrus after ram introduction, although melatonin did not modify cyclicity before the onset of the ram effect. This enhanced sensitivity of the hypothalamic GnRH pulse generator to the presence of the ram has been previously reported (Robinson et al., 1991). If the onset of the breeding season is a consequence of the number of long days experienced by the ewes (Malpaux et al., 1989), and if melatonin implants cause a short-day photoperiodic signal (O’Callaghan et al., 1991; Malpaux et al., 1997), it is possible that from spring Rasa Aragonesa ewes experienced a number of long or increasing days sufficient to respond to a short-day treatment. Sweeney et al. (1997) postulated that ewes are able to reinitiate reproductive activity in response to 35 long days followed by short days at any stage between the winter and summer solstices. The role of short and decreasing days between the autumn equinox and the winter solstice is to break the photorefractoriness to long days (Wayne et al., 1990). It has also been reported that melatonin treatment in spring and an associated male effect is able to increase fertility in French flocks (Chemineau et al., 1991).

The improvement of litter size induced by melatonin after implantation in spring is mainly due to an increased ovulation rate, as has been previously reported in both seasonal (Haresign, 1992; Robinson et al., 1991) and Mediterranean ewes (Forcada et al., 1995; Zúñiga et al., 2002). Some recent studies on synchronised ewes found melatonin treatment not to modify the timing of follicle emergence, but that it increases the number of ovulatory follicles by decreasing the atresia of medium and large follicles (Bister et al., 1999; Noël et al., 1999).

On the farm where ewes were treated with melatonin close to the winter solstice (Farm 1), reproductive performance was not improved in Rasa Aragonesa ewes. This is in contrast with previous results in the same breed, in which melatonin significantly improved both fertility and fecundity after implantation at approximately the
same time (29 December) (Forcada et al., 2002). These contradictory results are more remarkable considering that the flock used in the quoted work belonged to the same association and was located in the same village as the farm used in the present study—where control ewes exhibited an abnormally high fertility rate (90%) and was difficult to improve by melatonin implants. However, the pineal hormone was also unable to increase fertility after implantation in February—Farm 2, probably as photorefractoriness to short days was established.

Concerning the Assaf ewes, melatonin treatment induced a significant improvement in fertility after implantation in January and February (winter). The onset of the seasonal anestrous period was thought to be a consequence of the photorefractoriness to short days as perceived by the ewes before and after the winter solstice (Robinson and Karsch, 1984). However, Malpaux et al. (1988) reported the development of refractoriness to a short photoperiod to be temporarily overcome or delayed by exposure to a shorter day length. Therefore, it is possible that the Assaf ewes respond to melatonin treatment shortly after the winter solstice, before the full refractoriness to short days is established. Fertility exhibited by the control ewes was low, particularly those subsequent to the January and February (winter) treatment periods (31 and 20%, respectively), showing the deep sexual anoestrus of the breed. Although no data are available in the literature concerning the cyclicity of Assaf ewes during the seasonal anoestrous period, Palacios et al. (2002) reported Assaf females reared in Spain at the same latitude to have a higher sexual seasonality than other dairy sheep (Churra and Lacaune) reared in the same geographical area.

Fertility in Assaf ewes was also significantly improved by melatonin implantation after treatment in May (spring). In fact, it was evident that no treatment effect on litter size was recorded. Therefore, the improvement of the fecundity induced by melatonin in this breed was due only to the higher fertility in the treated ewes. This is a very interesting phenomenon of pineal gland secretion in dairy sheep—as the main objective to be reached in dairy livestock is increasing the number of lambings (and therefore of milking periods), to the detriment of the number of lambs.

Treatment of Merino ewes with melatonin only improved fecundity after treatment in February (winter), where 0.30 extra lambs per ewe treated were recorded as a consequence of an increase in both fertility and litter size. The fertility of ewes treated in April (spring) was also significantly higher than that recorded in non-treated females—although the latter showed a higher lambing rate (75%), exhibiting a reduced sexual seasonality in the Merino breed. A significant improvement in fertility after treatment in March (early spring) has been previously reported (Gómez et al., 1995). In spite of the low ovulation rate in the Merino, melatonin seems to improve litter size during the mating period after a treatment in February (winter). The main factor that limits ovulation rate in the Merino is the high rate of follicular atresia (Driancourt et al., 1990; López et al., 1997) and melatonin can improve the number of ovulatory follicles by decreasing the atresia of medium and large follicles (Bister et al., 1999; Noël et al., 1999). The pineal hormone can be a useful tool to increase productivity in Merino flocks.

Considering the particular conditions on the farms where this trial was carried out, implies that a farm effect should be considered. Melatonin was able to increase the overall number of lambs produced in the three breeds under study, although its efficacy seems to differ, considering the breed and time of treatment. Thus, it should be considered that the expected effects of melatonin can be achieved to a variable extent on commercial farms, provided that a realistic assessment of specific conditions on each farm is used to determine a treatment time (Kouimtzis et al., 1994). The most consistent results were obtained in the Assaf breed, with good results both in winter and spring melatonin treatment. Merino ewes only increased in reproductive performance after treatment in February (winter). The best results in the Rasa Aragonesa breed were obtained when melatonin implants were inserted in spring. It can be concluded that melatonin could be a useful tool to improve lamb production in the three breeds under study, although its efficacy within each breed seems to vary according to the farm and season.

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