Effect of melatonin implants on sexual activity in Mediterranean goat females without separation from males

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Abstract

This work was designed to determine whether melatonin treatment at the spring equinox can induce reproductive activity in goats without separation from males (separation being the normal practice in Spanish farming systems) and whether this treatment modifies the onset of the natural breeding season. Twenty-nine entire does were distributed into two groups (Group M, n = 14; Group C, n = 15). A third group of ovariectomized, estradiol-treated goats (OVX group, n = 5) was used to study the effect of melatonin on reproductive activity. On March 18, Groups M and OVX received a subcutaneous melatonin implant. In entire females, estrus was tested daily using entire aproned males, and ovulation rate was assessed after identification of estrus. Plasma progesterone in entire goats, plasma luteinizing hormone (LH) in the OVX group, and live weight and body condition score for all animals were recorded once a week. In entire goats, a clear treatment by time interaction was observed for progesterone concentrations (P < 0.001), with a period of high progesterone concentrations during the natural seasonal anestrus in Group M. A similar period of high LH concentrations was observed in the OVX group. Whereas all females of Group M presented ovarian activity during this period, no female of Group C did. The resumption of the natural breeding season was retarded in Group M in comparison with that in Group C (P < 0.05). We can conclude that in Mediterranean goats, melatonin implants can induce reproductive activity without separation from males, and it causes a small retardation in the reactivation of reproductive activity in the natural breeding season.

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1. Introduction

Some breeds of goats and sheep originating from mid and high latitudes exhibit a seasonal variation in reproductive activity during the year [1,2]. The timing of reproductive seasonality in these small ruminants, as well as in most mammals of the temperate zones, is controlled by photoperiod [2]. Photoperiod information is conveyed to the neuroendocrine system by the circadian secretion of melatonin from the pineal gland [3]. This hormone is synthesized and secreted by the pineal gland in a light-dependent daily rhythm generated by an endogenous circadian clock: high levels during the dark phase of a light-dark illumination cycle and low levels during the light phase [4,5].

Goats are denominated short-day breeders because their period of sexual activity occurs in the autumn to winter, and because the transfer to short days (8 h of light per day) and long days (16 h of light per day) is followed by a stimulation and an inhibition, respectively, of
reproductive activity [2]. As short days are characterized by a longer duration of melatonin secretion than that on long days, different attempts have been made to mimic the effect of short days by artificially prolonging the duration of high levels of circulating melatonin. Exogenously administered melatonin from continuous slow-release implants has been shown to advance the onset of the breeding season in sheep and goats by mimicking the stimulatory effect of short days [2,6] without suppressing the endogenous secretion [7,8]. However, although short days (induced by photoperiodic treatments or by exogenous melatonin) stimulate breeding activity, animals eventually become refractory to short days when kept under this day length for extended periods of time [2]. They cease their reproductive activity, reinitiating the breeding season after a period of sexual rest.

Traditionally, exogenous melatonin treatment is accompanied by a prior separation of males and females for 45 d, inducing a male effect to optimize the response during the anestrus period, obtaining a higher synchronization at mount. Male effect is a habitual practice in some extensive or semiextensive farm systems, but intensive systems generally present difficulties in separating males and females to induce this male effect, and hypothetically there could be more problems in using a melatonin treatment.

Mediterranean breeds have a short seasonal anestrus, especially when social (male effect) or nutritional factors are managed properly [9–11]. Therefore, in Mediterranean flocks at commercial level, melatonin implants are usually inserted at approximately the spring equinox [12,13].

The aim of this study was to determine whether treatment with exogenous melatonin at the spring equinox can induce reproductive activity in goats without the use of male effect (short-term effect), and whether this treatment causes a variation in the reactivation of reproductive activity in the natural breeding season (medium-term effect).

2. Materials and methods

2.1. Animals

This experiment was performed in accordance with the Spanish Animal Protection Policy RD1201/2005, which conforms to the European Union Directive 86/609 regarding the protection of animals used in scientific experiments.

The study was conducted on an experimental farm (latitude 37°15′N) of the University of Huelva, which meets the requirements of the European Community Commission for Scientific Procedure Establishments (1986). Twenty-nine entire, nonpregnant adult female goats, which were between 1.5 and 4 yr old at the beginning of the experiment and had kidded at least 5 months previously, were used. The experiment started on March 4, when the goats were assigned at random to the two experimental groups, balanced for live weight (LW) and body condition score (BCS) [14], and finished on September 30. Both groups—the control group (Group C, n = 15) and the melatonin-treated group (Group M, n = 14)—were kept in the same communal yard with an uncovered area throughout the experiment and without any supplementary light. On March 18, Group M received 1 subcutaneous melatonin implant containing 18 mg of the hormone (Melovine; CEVA Salud Animal, Barcelona, Spain) at the base of the left ear. These implants release melatonin for about 10 wk and raise daytime concentrations to about 100 pg/mL in goats [15] and in ewes [16].

In addition, a third group of ovariectomized, estradiol-treated goat does (OVX, n = 5) were implanted with exogenous melatonin at the same date as the entire females and, similarly to them, were kept in a communal yard with an uncovered area throughout the experiment and without any supplementary light. Prior to the start of the experiment, the does used in the OVX group were ovariectomized and implanted with a 3-cm silicone implant (internal diameter (i.d.) = 3.3 mm; external diameter (o.d.) = 4.6 mm) [17] containing crystalline estradiol (Sigma Chemical Co., St. Louis, MO, USA). Implants were soaked in physiologic serum before insertion to prevent an initial peak of steroid release. This group was used to study the direct effect of melatonin implant without the contact with the males to demonstrate whether melatonin alone implanted at this moment is able to induce the reactivation of luteinizing hormone (LH) secretion during the natural seasonal anestrus. For this reason, this group was completely isolated from the rest.

During the whole experiment, animals were maintained under intensive management and were fed daily with lucerne hay, barley straw ad libitum, and commercial concentrate, according to Institut National de la Recherche Agronomique (INRA) standards [18], to maintain adult weight or allow growth in younger females throughout the experiment. All animals had free access to water and mineral blocks containing trace elements and vitamins.

2.2. Sampling

To avoid a possible male effect that could modify the reproductive activity, before the onset of the experiment
bucks were allocated close to the intact females in a separate barn. In entire does, estrous activity was tested daily using 10 entire aproned bucks. The males were introduced into the intact females group from March 4 until the end of the experiment at 0900, and sexual behavior of the bucks and females was observed for three consecutive hours, from 0900 to 1200, during the study. During the rest of the day, bucks were isolated in their barn but close to the females. Females standing at mounting by the male were considered in estrus. Ovulation rate was evaluated by laparoscopy 7 d after positive identification of estrus. To determine the occurrence of silent ovulations and to confirm ovarian activity, blood samples were collected weekly from each animal by jugular venipuncture and assayed for progesterone. Immediately after collection, samples were centrifuged and plasma was stored at −20 °C until assay. To define the changes in LH secretion in ovariectomized goats, blood samples were collected weekly [19] from each animal by jugular venipuncture. After blood collection, samples were managed as mentioned previously. Live weight and BCS were recorded weekly for all animals.

2.3. Hormone assays

Plasma progesterone concentrations were assayed by radioimmunoassay, using the technique described by Terqui and Thimonier [20]. The sensitivity of the assay was 0.125 ng/mL. The intra-assay and interassay coefficients of variation were 6.4% and 4.4%, respectively.

Plasma LH concentrations were determined using double-antibody ELISA, as previously described in Faure et al. [21]. The sensitivity of the assay was 0.1 ng/mL. The intra-assay and interassay coefficients of variation of the control were 4.0% and 2.1%, respectively.

All hormonal analysis was performed at the hormonal analysis laboratory of INRA (Nouzilly, France).

2.4. Definitions of reproductive activity

The reproductive activity induced by melatonin treatment (estrous activity or ovulatory activity) was defined as the first estrus or the first ovarian activity, respectively, after implant insertion, detected during spring, the natural nonbreeding season at this latitude. The reactivation of reproductive activity in the natural breeding season (estrous activity or ovulatory activity) was defined as the first estrus or the first ovarian activity, respectively, detected during the natural breeding season. The nonbreeding season and the natural breeding season were defined according to the results obtained at the same latitude and with a similar method by Zarazaga et al. [10]. For Group M, the mean duration of anestrus or of the anovulatory season was defined as the number of days between the last detected estrus or last ovulation induced by the melatonin treatment and the first detected estrus or first ovulation of the following breeding season [2].

Ovulatory activity was confirmed when two or more consecutive plasma samples had progesterone concentrations above baseline (0.5 ng/mL [22]) with subsequent cyclicity. For each goat, the date of the last plasma progesterone value below baseline that was followed by the first extended cyclic pattern was taken as the onset of ovulatory activity. Cessation of ovulatory activity was considered to have occurred when three or more consecutive plasma samples had concentrations below baseline. The date of the first plasma progesterone value below baseline at the completion of the last extended cyclic progesterone pattern was taken as the end of ovulatory activity.

Sexual activities observed during the experiment were grouped as (i) estrous cycle, when detected estrous behavior was accompanied by an increase in serum progesterone levels above 0.5 ng/mL; (ii) ovulatory activity, when an increase in serum progesterone levels above 0.5 ng/mL in at least two consecutive samples occurred but was not preceded by detected estrous behavior; (iii) ovulation rate of each detected cycle; (iv) length of estrous cycle classified as normal (16 to 27 days), short (<16 days), or long (>27 days) duration.

The reproductive state in the ovariectomized, estradiol-treated group was assessed using characteristics of the LH profile. The onset of pituitary activity was defined as the date of the first plasma LH sample above 0.5 ng/mL in a sequence of two or more samples above 0.5 ng/mL. The end of pituitary activity was defined as the date of the last plasma LH sample above 0.5 ng/mL, followed by a sequence of two or more points below 0.5 ng/mL [10].

2.5. Statistical analysis

Weekly means (±SEM) of LW, BCS, progesterone, and LH for each group were calculated. The effect of the treatment and time on LW, BCS, and progesterone was analyzed using an analysis for repeated measures. In entire goats, the date of the first detected estrus or first ovulatory activity induced by the melatonin implant during the seasonal anestrus, the date of the first detected estrus or the first ovulatory activity in the natural breeding season, and the ovulation rate of each
detected estrus in each period were determined for each goat. ANOVA was used to evaluate the effect of treatment on these reproductive parameters. The percentage of estrous cycles classified according to their duration and the incidence of ovarian activity and estrus induced by melatonin implant detected during the seasonal anestrus or during the natural breeding season were compared between groups using the chi-square test.

To study the effect of LW or BCS on ovulation rate at each detected estrus, entire goats were divided into groups depending on their LW at each estrus, Group 1 (LW ≤ 50 kg) and Group 2 (LW > 50 kg), or, depending on their BCS at the same time, Group A (BCS ≤ 2.50) and Group B (BCS ≥ 2.75).

In ovariectomized goats, the weekly mean (±SEM) effect of time on LH concentrations was analyzed using an analysis of repeated measures.

3. Results

3.1. Effect of treatment on live weight and body condition score

Fig. 1 shows the changes in LW and BCS during the experiment. No effect of melatonin treatment on LW was observed. At the start of the experiment, Groups M and C had similar LW and BCS. However, there was a clear effect of time (P < 0.001) and a treatment by
time interaction (P < 0.05). In Group M, LW was relatively stable during the nonbreeding season (defined as the time between the first and the last ovarian activity during the natural seasonal anestrus), when they gained 0.53 ± 26.54 g/d, and markedly increased during the period that they were reproductively inactive (this was defined as the period between the last ovarian activity during the natural seasonal anestrus and the first ovarian activity in the new breeding season), when they gained 34.98 ± 11.69 g/d. On the other hand, in Group C, LW increased from the onset of the experiment until the onset of the natural breeding season (47.92 ± 10.42 g/d).

With regard to BCS, no effect of treatment or interaction was observed, but there was a clear effect of time (P < 0.001).

3.2. Effect of treatment on progesterone and LH concentrations

Fig. 2 shows the changes in progesterone and LH concentrations during the experiment. Progesterone concentrations showed a clear effect of time and a treatment by time interaction (P < 0.001). The interaction demonstrated that exogenous melatonin induces a marked ovarian activity in the treated group during the natural seasonal anestrus. However, no effect of treatment was observed.

Group M showed two periods of high progesterone concentrations. The mean date of the first ovarian activity was April 17 (±3.20 d), with a range for the onset of ovarian activity from April 8 until May 13; the range for the end of ovarian activity was from May 20 until July 1. The progesterone values remained higher than baseline for 54 ± 4.95 d, until June 11 (±3.68 d). Furthermore, this group was characterized by 100% of the animals presenting ovarian activity in response to the melatonin treatment and 71.4% showing the first ovarian activity in the first 33 d after implant insertion. There is a clear, rapid effect of the melatonin implant in stimulating the progesterone concentrations, because after only 31.5 ± 2.82 d after implant insertion, the progesterone concentrations rose above baseline. The second period was from September 7 (±3.19 d) until the end of the experiment.

In contrast, the control group showed a period of increased progesterone concentrations related only to the natural breeding season, indicating that no female of this group presented ovarian activity during the natural seasonal anestrus, despite the animals being in contact with Group M. The period of high progesterone concentrations was from August 20 (±6.97 d) until the end of the experiment. Group C resumed the natural breeding season at an earlier date than that observed for Group M (P < 0.05). No differences between Groups M and C in the natural reactivation of the ovarian activity

![Fig. 2. Weekly mean (±SEM) for progesterone concentrations (ng/mL) of entire Mediterranean goat females subjected to natural changes in day length (Group C, □) or treated with exogenous melatonin on March 18 (Group M, ●) and for LH concentrations (ng/mL) in ovariectomized, estradiol-treated females implanted with exogenous melatonin on March 18 (O VX group, △). *P < 0.05, **P < 0.01.](image-url)
measured by progesterone concentrations were observed at any date. With regard to the LH concentrations, there was a significant effect of time (Fig. 2). The evolution of LH concentration in the OVX group was very similar to that of the progesterone concentrations in the entire goats of Group M, and no statistical differences were observed (P > 0.05). This group showed two periods of high LH concentrations (higher than 0.5 ng/mL): the first started on April 17 (±10.17 d), with values remaining high until June 5 (±9.3 d); the second period was from September 11 (±12.35 d) until the end of the experiment. Similarly to the case of Group M, the LH secretion increased rapidly after implant insertion, with a delay of only 30.33 ±10.17 d.

The results of the onset and the end of reproductive activity according to the LH or the progesterone concentrations in Groups OVX and M, respectively, were not different (P > 0.05). The increase of the LH secretion on the OVX group during the seasonal anestrus demonstrates that melatonin implant itself is able to induce a reproductive activity during this period and indicates that no contact with males is necessary. Similarly, no differences were observed for the onset of the natural breeding season between the two groups treated with melatonin (OVX and M).

### 3.3. Effect of treatment on date of onset of estrous activity in entire goats

As with the progesterone concentrations, no estrous activity was observed in entire goats of Group C during the natural seasonal anestrus, despite the animals being in contact with those of Group M. However, in Group M, 85.7% of the animals showed estrous activity, and 57.1% showed the first estrous activity in the first 33 d after implant insertion. The mean date of estrous activity for Group M during the seasonal anestrus was April 20 (±5.02 d). The dates of the reactivation of estrous activity in the natural breeding season were September 11 (±3.31 d) versus August 22 (±6.12 d) for Groups M and C, respectively (P < 0.01). The range of the onset of ovarian activity was from August 26 until September 27 and July 8 until September 23 for Groups M and C, respectively.

### 3.4. Effect of treatment on ovulation rate and duration of the cycle

The ovulation rate of the first detected estrus during the nonbreeding season in Group M was 1.60 ± 0.20 corpora lutea. No effect of treatment on this parameter was observed for the first estrus of the natural breeding season (1.42 ± 0.15 vs. 1.58 ± 0.19 corpora lutea for Groups M and C, respectively). No effect or interaction between treatment and the categories of LW or BCS was observed.

Table 1 shows the frequency distribution of cycles according to their length during the whole experiment. The proportion of short and long cycles was similar, and they were less frequent than normal cycles, with no observable differences between groups. With regard to the first estrus detected during the seasonal anestrus in Group M, only 14.23% were silent ovulations, and 21.4% were short cycles. However, at the resumption of the natural breeding season, only one short cycle at the first detected estrus was found in Group C.

### 4. Discussion

The results of the current study demonstrated that the treatment with exogenous melatonin at the spring equinox without separation from the male effectively induces reproductive activity during the natural seasonal anestrus (short-term effect) with normal cycles and sexual behavior, and that this treatment induces a small retardation in the reactivation of reproductive activity in the natural breeding season (medium-term...
effect). Moreover, there is no effect of the LW and BCS on this activity.

A pattern of body weight changes, associated with reproductive activity, was observed in all groups, despite the fact that they were fed to maintain their body weight; such variations in body weight have been reported previously in this species [10,11,23,24]. These changes occurred differently between Groups M and C, due to the treatment by time interaction. These results do not seem to be a direct effect of the action of photoperiod, as has been suggested by Barenton et al. [25]; rather it seems due to the fact that food intake was reduced [26], and probably there was greater activity as a consequence of an intense sexual activity, as reported in Australian cashmere bucks [27].

The LH pattern of the ovariectomized-implanted does clearly reflects the seasonal reproductive activity of the melatonin-treated entire does and contrasts with that of the control group. The LH and progesterone concentrations of the treated groups showed a marked effect of treatment with melatonin during the non-breeding season. These results demonstrate the effectiveness of inserting melatonin implants at the spring equinox at Spanish latitudes. Furthermore, they indicate that the period of increasing days between the winter solstice and the spring equinox could be necessary to induce reproductive activity in this period, as in sheep [12], although it has been shown that at higher latitudes the melatonin implant has to be inserted later in the year, nearer to the summer solstice [28]. The results in the OVX group contrast with those reported by Forcada et al. [29]. Those authors observed in ovariectomized, estradiol-treated ewes that the melatonin implant inserted at a date similar to that in our experiment was unable to induce a resumption of reproductive activity before the onset of the natural breeding season. This suggests that goats and sheep may differ in their sensitivity to photoperiod or melatonin implants.

The increase of LH or progesterone concentrations during the sexual rest was very soon after insertion of the melatonin implant in treated groups. The difference is very high if we compare with the results of Chemineau et al. [30] who did not observe any ovulation before the introduction of males after 71 d of melatonin treatment. The increase in the mean values of LH has also been attributed to the effect of melatonin on the hypothalamus-hypophysis [31], increasing the secretion of LH. Afterwards, LH and progesterone concentrations in the treated groups fell to the basal level, probably because of refractoriness to the stimulatory short-days signal provided by melatonin [32,33].

It is important to note that a clear reproductive activity with a very high percentage of response to the melatonin treatment was obtained without isolation from the males. To our knowledge, our findings are the first of this type observed in melatonin-implanted goats, as the male effect is a normal practice when melatonin implants are used. In the References list, the only results from a study of the effectiveness of melatonin implants without separation of the males are those obtained in ewes [34]. The treated ewes showed no reproductive activity during the seasonal anestrus, but the melatonin implants were inserted 1 mo later than in our experiment; they did, however, exhibit an earlier onset of the natural breeding season.

Regarding the percentage of the response in treated goats, our result is similar to that obtained by Pellicer-Rubio et al. [35] working with Alpine goats at a higher latitude and in the Damascus goat breed treated with melatonin in May (spring) [36] in both cases with male effect. However, Chemineau et al. [2], working with Alpine goats treated only with melatonin, obtained a cyclicity of only 46% after the introduction of the males. However, our results in the control group contrast with previous ones obtained by Restall et al. [37], who showed that estrous females can induce ovulation in anovulatory does—they obtained 83% of estrus in anovulatory does exposed to induced-estrus females. This influence of the female had not been studied previously in Mediterranean goats.

The reactivation of the ovarian and estrous activity in the entire goats and the LH secretion in the OVX group in the natural breeding season, despite the retardation observed in treated goats, was similar to that described by Zarazaga et al. [10]. This retardation could be due to the melatonin treatment. The time of reproductive onset in autumn depends on the time of exposure to long days after the winter solstice [38]. The more delayed the exposure to long days, the more delayed the onset of reproductive activity in implanted animals, because the exhaustion of the melatonin implant delayed the perception of the natural long days.

The absence of effect of the melatonin implant on ovulation rate is similar to that reported in ewes by McEvoy et al. [39] and contrasts with previous results [34,40]. Similarly, there was no interaction of BCS or LW with melatonin treatment for the ovulation rate. To our knowledge, our findings are the first of this type observed in melatonin-implanted goats.

In conclusion, the results of the current experiment support the hypothesis that Mediterranean goat does treated with exogenous melatonin and without separation from males showed a clear reproductive activity
during the nonbreeding season, presenting cycles of normal duration with normal estrus, and this treatment causes a slight retardation in the reactivation of reproductive activity in the natural breeding season (medium-term effect). Moreover, there is no effect of the LW or BCS on this activity or the ovulation rate.

Conflict of interest statement

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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