Induction of sexual activity in lactating anovulatory female goats using male goats treated only with artificially long days[1]

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ABSTRACT: Two experiments were conducted to determine the response of Creole male goats treated with long days and melatonin implants, and the response of the anovulatory does to male effect using males treated only with artificially long days. All animals were allocated to open sheds. In Exp. 1, one group of males was under natural photoperiod (CG; n = 7); the second group was submitted to 2.5 mo of long days followed by the insertion of two s.c. melatonin implants (LD+MEL; n = 7); the third group was subjected only to 2.5 mo of long days (LD; n = 7). Testicular weight was measured every 2 wk. Plasma testosterone concentrations were determined weekly. A treatment × time interaction was detected ($P < 0.001$) for testicular weight and plasma testosterone concentration. In the LD+MEL and LD groups, testicular size and plasma testosterone levels varied in a similar way, but differed from those observed in CG ($P < 0.001$). In this latter group, testicular weight displayed seasonal variations and peaked in June, whereas in treated groups this peak occurred in March. In CG, testosterone varied in a seasonal manner and plasma concentrations increased in June and remained elevated throughout the study. In experimental groups, testosterone increased in February and peaked in March. In Exp. 2, one group of males was left under natural photoperiod (CG, n = 5) and the other one was submitted to 2.5 mo of artificially long days (LD, n = 4). On March 16, two control and two treated males were put in contact with 20 and 19 females, respectively. Sexual behavior of the bucks was observed during the 5 d following male introduction. Progesterone assays and estrous behavior were used to determine ovarian and behavioral responses of the females to teasing. The anogenital sniffing, nudging, and mount instances registered in LD-treated males were greater than those observed in CG ($P < 0.05$). Of the does exposed to CG, none ovulated and only two of 20 females displayed estrous behavior. All does in contact with LD-treated males ovulated and showed at least one estrous behavior during the 15 d following joining ($P < 0.001$). These results indicate that the sexual activity of male goats from subtropical latitudes can be induced using only artificially long days. In addition, males treated in this way are capable of stimulating sexual activity in anovulatory females by the male effect.

Key Words: Goats, Melatonin, Photoperiod, Sexual Behavior

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Introduction

Reproductive seasonality is a major limitation in goat production in subtropical and high latitudes (Delgadillo et al., 2000). Various treatments have been proposed to control this phenomenon, including male effect or photoperiod (Chemineau et al., 1999). In seasonally anovulatory does in the subtropics, the use of males receiving a photoperiodic treatment is sufficient to induce ovulation in most females after teasing, whereas untreated males are unable to do so (Flores et al., 2000). The photoperiodic treatment consisted of a sequence of long days for 2.5 mo followed by short days (Chemineau

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et al., 1992). Under field conditions, the long-day part of the treatment is easy to apply as extra illumination can be provided indoors or outdoors. In contrast, short days are more problematic as exposure to artificial short days is not compatible with most management systems. Short days may be replaced by the use of a melatonin implant that provides a short-day signal (Donavan et al., 1994). Whether these artificially short days play a critical role in the efficiency of the treatment in male goats is not obvious. Indeed, long days play a critical role in natural conditions to time the onset of the breeding season by phase-shifting the annual reproductive rhythm (Malpaux et al., 1989; Barrell et al., 2000). Long days applied in winter may therefore be sufficient to induce out-of-season breeding. In addition, when melatonin is applied in these treatments, ambient day length, although increasing, is rather short and a decrease from artificially long days to this shorter day length may provide a sufficient stimulatory signal reinforcing the initial effect of long days (Robinson and Karsch, 1987). The aim of this study was therefore to determine whether, following a treatment with long days in November through January, the insertion of a melatonin implant is required to stimulate sexual activity in male goats and to make them able to induce ovulation after teasing in anovulatory females.

Materials and Methods

General. In both experiments, male goats were part of a homogeneous group of adult Creole males from the Laguna region in the State of Coahuila, Mexico (26°N). The population of local animals, called Criollo, is variable from a phenotypic and coat-color viewpoint. Under extensive management conditions, the mean body weight of adult males and females is about 45 and 35 kg, respectively (Delgadillo et al., 1999). Males were given ad libitum access to alfalfa hay (18% CP), water, and mineral blocks, and 300 g of commercial concentrate (14% CP; 1.7 Mcal/kg).

Experiment 1

The objective of Exp. 1 was to determine if melatonin is necessary to stimulate testosterone secretion and testicular weight.

Photoperiodic Treatments. Three groups of animals were used in this experiment. Two groups of animals were exposed to long days for 2.5 mo between November 1, 1998 and January 15, 1999. Thereafter, both groups were exposed back to the natural variations in day length. On January 16, bucks in one long-day group (n = 7) received two s.c. ear melatonin implants (18 mg each; Regulin-Mélovine CEVA Santé Animale, Libourne, France). Briefly, these implants release melatonin for about 10 wk and elevate daytime concentrations to about 100 pg/mL in ewes (Staples et al., 1991). As previously validated in our animal model (Delgadillo et al., 2001), two implants were inserted to take into account the size of the animals. This treatment was shown to stimulate testosterone secretion and improve sexual behavior during the nonbreeding season (Delgadillo et al., 2001; Véliz et al., 2002). The other long-day group (n = 7) experienced long days but did not receive melatonin, in order to test whether melatonin is needed to obtain full stimulation in males. The third group (n = 7) remained in natural photoperiod throughout the experiment. Day length varied from 13 h 41 min at the summer solstice to 10 h 19 min at the winter solstice. All bucks in each group remained together in a separate 5- × 7-m open shed under natural day length and ambient-temperature conditions. Long days were provided to the animals in the open shed; artificial light was given from 0600 to 0800 and from 1800 to 2200 to extend the natural day (i.e., in order to obtain a total of 16 h of light/d). Photoperiod was regulated by an electric clock and light intensity was at least 300 lx, positioned laterally to the eyes of the animals.

Measurements. Body and testicular weight were determined every 2 wk throughout the study which lasted until October 30, 1999. Testicular weight was assessed by comparative palpation with an orchidometer (Oldham et al., 1978). The determinations were carried out by the same operator throughout the study. Plasma testosterone concentrations were determined in blood samples obtained at 0800 once each week throughout the experimental period.

Experiment 2

The objective of Exp. 2 was to determine if a long-day treatment is sufficient to induce sexual behavior in males and make them capable of stimulating estrous behavior in anestrous females.

Photoperiodic Treatments. Two groups of males were used in this experiment. A control group (n = 5) was exposed to natural photoperiod while an experimental group (n = 4) received the same treatment as the long-day group of the first experiment (natural photoperiod except for exposure to long days between November 1, 1999 and January 15, 2000). They were used to perform the male effect on March 15, 2000.

Preparation of Females Used to Test the Teasing Ability of Males. Multiparous Mexican Creole goats (n = 56) belonging to the same flock were maintained under extensive management system before the beginning of the study. They had given birth between October and December 1999, and all females were hand-milked once a day throughout the study. On February 23, 2000, the females were kept in shaded 7- × 10-m pens with nine to 10 females in each and were given ad libitum access to water, mineral blocks, and alfalfa hay (18% CP), and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg). On February 24 and March 5, 2000, plasma progesterone concentrations were measured in blood samples by RIA to determine ovarian cyclicity (Terqui and Thimonier, 1974). Seventeen does showed ovarian activity and were removed from the experiment. As a consequence,

39 females were used. On the day of teasing (d 0), another blood sample was collected from each doe to check that no other females had initiated ovulatory activity.

Organization of Male Effect and Measurements. Before teasing, females were not allowed contact with males for 2.5 mo, and bucks were kept on another experimental farm 15 km from females. On March 15, 2000, two control and two long-day treated males chosen at random were put in contact with 20 and 19 females, respectively. The female groups were balanced for parturition date, body weight, and milk production. These constituted the control and long-day group, respectively. The two groups of females were separated from each other by about 120 m. During the first 5 d following the introduction of males, sexual behavior of bucks was observed by four trained persons for 2 h, from 0800 to 1000. Each person observed each buck individually and recorded the following characteristics: ano-genital sniffing, nudging, mount intention movements, and mounts (with and without ejaculation).

Detection of Estrous Behaviour. Males were fitted with marking harnesses. From March 15 to March 20, estrous behavior was recorded by direct visual observation every day between 0800 and 1000 and between 1700 and 1900, using the control and long-day treated bucks. During these first 5 d, immobilization of female at mounting by the male was considered a sign of estrous behavior (Mauléon and Dauzier, 1965). The females marked by bucks also were considerd in estrus. From March 21 until the end of experiment on March 30, estrous behavior was recorded twice daily (0800 and 1800) by visual observation of marks from raddled bucks (Walkden-Brown et al., 1993).

Ovarian Activity. Plasma progesterone concentrations were determined in blood samples obtained daily from d 1 to d 15, after introduction of the males.

Blood Samplings. All blood samples were collected by jugular venipuncture in tubes containing EDTA. Plasma was obtained after centrifugation at 2,500 × *g* for 20 min and stored at –20°C until hormone concentrations were measured. Plasma testosterone was measured by RIA in duplicate, in 50-μL plasma samples as described by Garnier et al. (1978). Sensitivity (2 SD from zero control) was 0.1 ng/mL. The intraassay CV was 8%. Concentrations of plasma progesterone were measured by RIA as described by Saumande et al. (1985). Sensivity was 0.1 ng/mL. The intra- and interassay CV were 7 and 11%, respectively. Females with progesterone levels above 0.5 ng/mL were considered to have ovulated (Gómez-Brunet et al., 1995).

Statistical Analyses. Effects of treatment on BW, testicular weight, and plasma testosterone concentrations were submitted to ANOVA for repeated measures. When treatment by time interactions were detected, treatment effects were examined within time using pairwise comparisons among treatments. Differences in the frequencies of male sexual behaviors recorded in both groups were analyzed using χ^2. The proportions of females showing estrous behavior were compared by a Fisher exact probability test. Analyses were computed using SuperANOVA and StatView (Abacus Concepts Inc., Berkeley, CA).

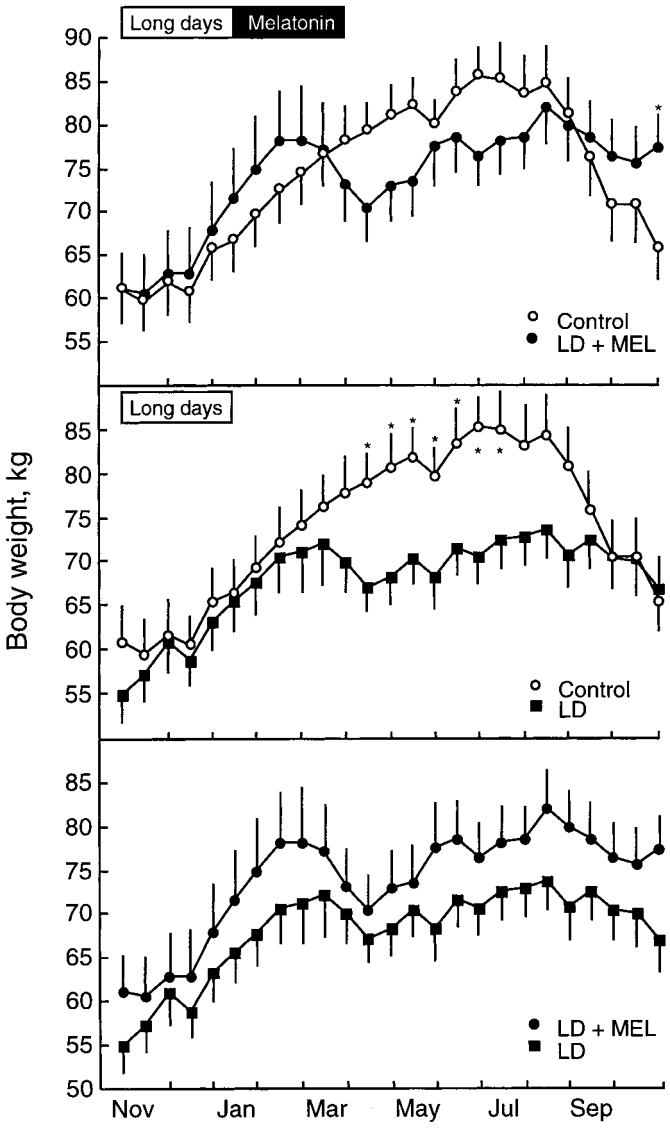

Figure 1. Exp. 1. Body weight (mean ± SEM) in three groups of male Creole goats subjected to natural changes in day length (open symbols); to 2.5 mo of artificially long days between November 1 and January 15, and then treated with two s.c. melatonin implants (closed circle symbols); or 2.5 mo of artificially long days followed by natural short days (square symbols). Body weight was determined twice a month. Stars indicate significant differences between groups (*$P < 0.05$).

Results

Experiment 1

Body Weight. A treatment by time interaction was detected ($P < 0.001$) for BW, necessitating examination of treatment effects within time (Figure 1). Pairwise comparisons revealed that BW did not differ ($P > 0.10$)

between the two long-day groups. In contrast, BW in these two groups differed ($P < 0.01$) from those in bucks exposed to natural photoperiods. In control bucks, BW started to increase progressively from December and peaked in June. Then BW decreased until the end of measurements. In both treated groups, BW peaked on February 30 (long-day + melatonin group), and on March 15 (long-day group), 1.5 and 2 mo after the long days treatment ended. Thereafter, BW stabilized without large variations throughout the study.

Testicular Weight. Testicular weight in control and experimental groups is shown in Figure 2. A treatment by time interaction was detected ($P < 0.001$) for testicular weight. Pairwise comparisons revealed that testicular size did not differ ($P > 0.10$) between the two long-day groups. In contrast, testicular weight in these two groups differed ($P < 0.001$) from those in bucks exposed to natural photoperiod. In control bucks, testicular weight displayed seasonal variations, and as expected, testicular size started to increase progressively from January and peaked on June 15. Testicular weight then decreased from July and reached a minimum on October 15. On the contrary, in both experimental groups, testicular size peaked on March 30 (i.e., 2.5 mo after long-day treatments ended). Afterwards, testicular weight declined sharply in March, 6 wk after the cessation of long-day treatments, reaching lower values on May 30.

Patterns of Testosterone Secretion. A treatment by time interaction was detected ($P < 0.001$) for plasma testosterone. Testosterone values did not differ ($P > 0.10$) between the two long-day groups. However numerous differences were found when the long-day bucks were compared with bucks exposed to natural photoperiod (Figure 3). In the control group, plasma testosterone concentrations displayed seasonal variations with the lower concentrations (< 5 ng/mL) occurring between December and May. Then, an increase occurred in June, and concentrations remained elevated throughout the study. In both long-day groups, testosterone concentrations increased dramatically as early as February and peaked on March 6 (long-day group) and 13 (long-day + melatonin group), at a level observed during the natural breeding season in the control group. Testosterone concentrations then decreased progressively until mid-July. At the end of July, they rose again, reaching concentrations similar to those of the control group.

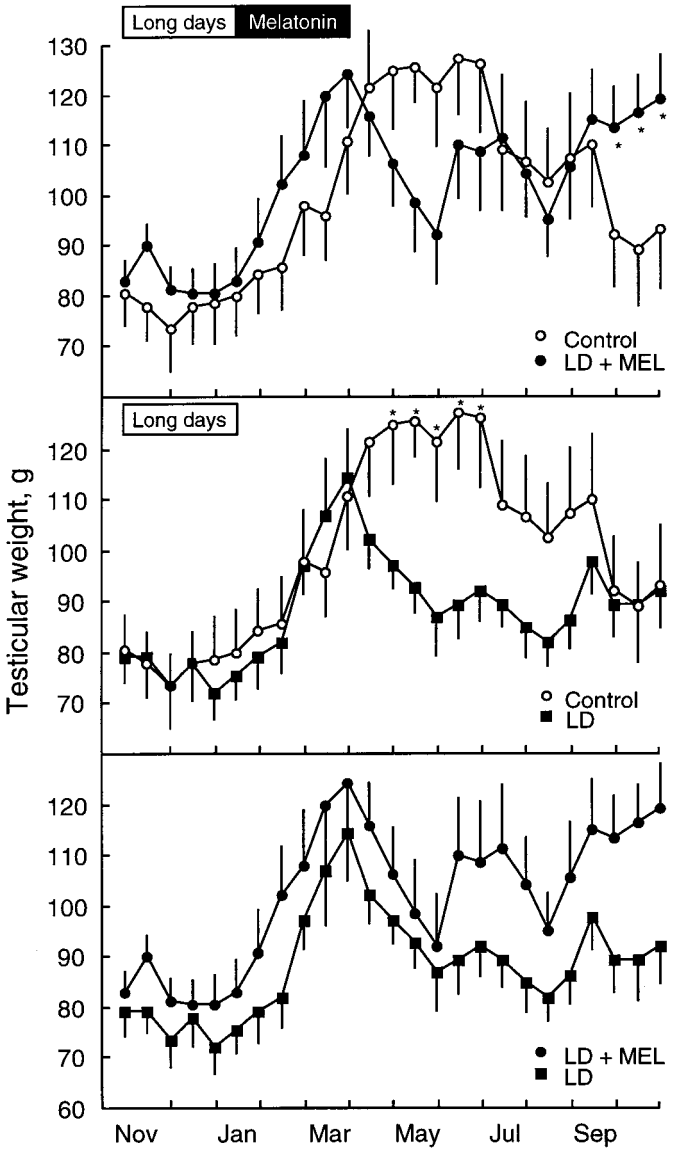

Figure 2. Exp. 1. Testicular weight (mean ± SEM) in three groups of male Creole goats subjected to natural changes in day length (open symbols); to 2.5 mo of artificially long days between November 1 and January 15, and then treated with two s.c. melatonin implants (closed circle symbols); or 2.5 mo of artificially long days followed by natural short days (square symbols). Testicular weight was determined twice a month. Stars indicate significant differences between groups (*$P < 0.05$).

Experiment 2

Sexual Behavior of Bucks. The sexual behavior of bucks during the first 5 d after being joined with females differed between the control and long-day treated group (Figure 4). Of the 325 anogenital sniffing instances observed, 18 were performed by the control bucks and 307 by the long-day treated males ($P < 0.001$). Of 231 instances of nudging observed in both groups, seven were performed by the untreated males and 224 by the treated bucks ($P < 0.001$). Of the 12 mounts observed, 10 were performed by the long-day treated bucks ($P < 0.05$). Finally, the mount-intention movement numbers were not different between groups, and all events (three instances) were performed by the treated males.

Response of the Females to the Male Effect: Ovarian and Estrous Activity. No females exposed to control bucks ovulated and only two of 20 females were detected in estrus 3 and 4 d after male introduction. In contrast, 19 of 19 females exposed to the long-day treated males ovulated and showed an estrous behavior during the 11-d period following male introduction ($P < 0.001$). In

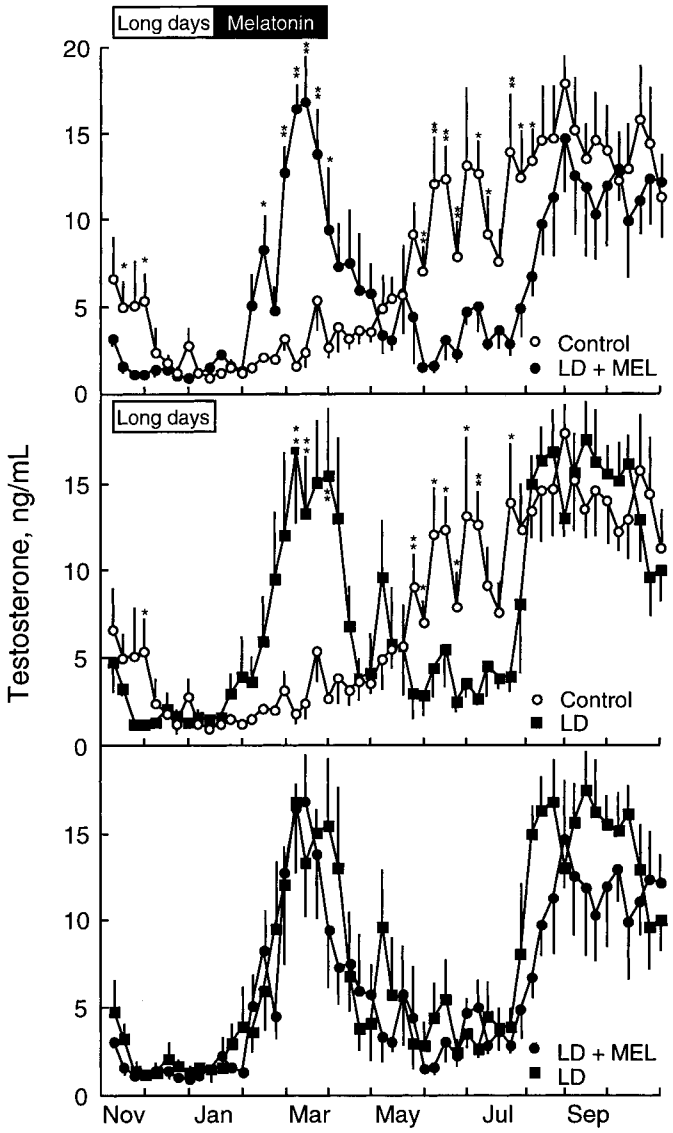

Figure 3. Exp. 1. Plasma testosterone concentrations (mean ± SEM) in three groups of male Creole goats subjected to natural changes in day length (open symbols); to 2.5 mo of artificially long days between November 1 and January 15, and then treated with two s.c. melatonin implants (closed circle symbols); or 2.5 mo of artificially long days followed by natural short days (square symbols). Blood samples were taken once a week. Stars indicate significant differences between groups (*$P < 0.05$; **$P < 0.001$).

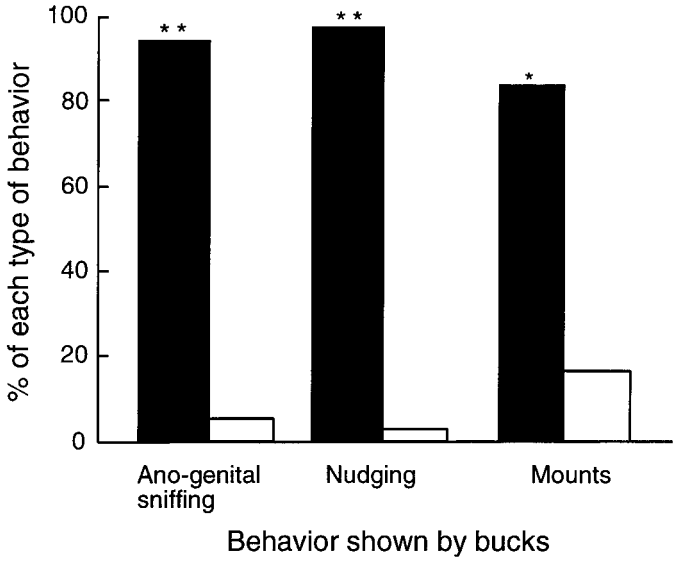

Figure 4. Exp. 2. Distribution of each type of behavior observed between two groups of males, expressed as a percentage of the total number of behavior characteristics. Sexual behavior was recorded for 2 h daily during the first 5 d of teasing with does of Exp. 2. The control males were exposed to natural changes in day length (open bars; n = 2), while long-day treated males were subjected to 2.5 mo of artificially long days between November 1 and January 15, followed by natural short days (solid bars; n = 2; *$P < 0.05$; **$P < 0.001$).

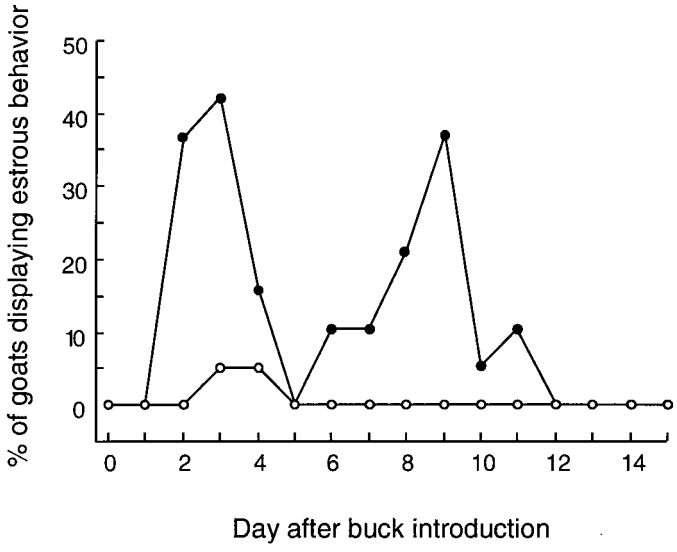

Figure 5. Exp. 2. All females in a group of seasonally anovulatory lactating goats displayed estrous activity after introduction of long-day treated males, which were subjected to 2.5 mo of artificially long days between November 1 and January 15, followed by natural short days (solid circles; n = 19). Estrus was observed in only two goats teased with control males subjected to natural changes in day length (open circles, n = 20). Day 0 is day of teasing.

this group, 18 of 19 females displayed estrous behavior within the first 4 d after male introduction, and 1 did so 5 d later. Seventeen of 19 goats showed a short estrous cycle of 5.2 ± 0.4 d duration and displayed a second estrus between 6 and 11 d after teasing. The interval between the introduction of males and the onset of estrous behavior was 3.2 ± 0.4 d (Figure 5).

Discussion

Results demonstrate that in northern Mexico, local male goats treated with long days at the end of the

normal breeding season show a clear stimulation of testosterone secretion in the same way bucks treated with both long days and melatonin do. In addition, this photoperiodic treatment causes a stimulation of various male sexual behaviors that make these males capable of inducing estrous behavior in a high proportion of females exposed to them.

The results obtained in the group of males submitted to long days and melatonin confirm that this photoperiodic treatment stimulates testosterone secretion during the rest season in males adapted to a subtropical environment (Delgadillo et al., 2001). The main outcome of this study is the demonstration that exogenous melatonin is not necessary to stimulate endocrine activity in bucks undergoing this type of treatment. Indeed, the response to photoperiodic treatment assessed by testosterone secretion indicates that the pattern of this hormone did not differ between long-day + melatonin and long-day treated males. In both groups, testosterone secretion was stimulated during the period of low secretion in control animals. Testicular weights did not show differences between the two long-day treated groups, and they also did not differ from those of controls between November 1 to March 30, 2.5 mo after the long-day treatment ended. The absence of differences in testicular weight during this period in the present study and those carried out in our laboratory by F.G. Véliz (unpublished data), could result from the effects of photoperiodic treatment upon testosterone secretion and food intake. In control bucks, an increase in food intake during the nonbreeding season can increase BW and testicular weight independently of LH secretion (Walkden-Brown et al., 1994a,b; Delgadillo et al., 1999). During the breeding season, food intake decreases (probably as a consequence of increased testosterone secretion) resulting in reduced BW, and as consequence, testicular weight, as reported in Australian cashmere bucks and Soay rams (Walkden-Brown et al., 1997; Lincoln, 2001). In long-day treated groups, the photoperiod treatment stimulated testosterone secretion between February and March, which probably caused a decrease in food intake, BW, and a decline in testicular weight. Moreover, the acute rises in plasma testosterone concentrations in long-day treated bucks probably enhanced the inhibition of LH release by testosterone negative feedback, which also may have participated in the reduction in testicular size (Delgadillo and Chemineau, 1992; Araki et al., 2000). This could explain why the differences in testicular weight reported previously in the same long-day + melatonin-treated bucks were not found in this study (Delgadillo et al., 2001). The fertility of does mated with treated males using the male effect is about 60% (Flores et al., 2000), suggesting the males have good quantitative and qualitative sperm production.

Data obtained in Exp. 2 clearly indicate that exogenous melatonin is not necessary to make the males fully able to induce ovulation in females after teasing. Indeed, the male goats treated only with 2.5 mo of long days displayed a much higher sexual behavior than untreated bucks. Anogenital sniffing, nudging, and mount instances were greater in long-day treated bucks than in control males when exposed to anestrous does. These males were fully capable of inducing the estrous behavior of does during the anestrous period. Of the does in contact with control males, none ovulated and only 10% were detected in estrus, but all females in contact with long-day treated males ovulated and displayed at least one estrous behavior during the study. These data confirm our previous results that at least in our subtropical conditions, male reproductive condition is an important factor that determines the quality of response in females to the male effect. Females respond to teasing only when sexually active males are used (Flores et al., 2000; Véliz et al., 2002). Interestingly, preliminary results obtained in our laboratory suggest that the long-day treated bucks are able to stimulate the sexual activity of female goats maintained in extensive conditions (J.A. Delgadillo, unpublished data).

The observation that treatment with long days only is sufficient to stimulate the sexual behavior of the males and that subsequent melatonin treatment is not necessary can be explained by two nonexclusive hypotheses. Firstly, in sheep, it is well demonstrated that the seasonal variations in reproductive activity are, for the most part, the expression of an endogenous rhythm and that the perception of long days in the spring is a critical synchronizing signal of the rhythm that is involved in timing the onset of the breeding season (Woodfill et al., 1994; Malpaux et al., 2001). Although not demonstrated in goats, this mechanism is probably operating in the same way as in sheep. Therefore, the exposure of the animals to long days in November through January (i.e., much earlier than in natural conditions) most likely causes a phase advance of the reproductive rhythm. Secondly, the drop from artificially long days (16 h of light/d) to the natural short days of January (about 10 h 19 min of light/d) caused an acute stimulation of reproductive activity. However, it must be noted that, after this initial stimulation from January to March, day length increased progressively and therefore provided a potentially inhibitory signal (Malpaux et al., 1989). The absence of difference in testosterone secretion between animals exposed to natural photoperiod or treated with melatonin after the long-day treatment indicates that this inhibitory signal could not cancel the stimulation obtained by the phase shifting of the endogenous rhythm and the initial stimulation caused by the drop in photoperiod. This observation was made in subtropical conditions where the change in day length between winter solstice and the spring equinox is about 1 h 40 min, and it would be interesting to determine whether it is still valid in higher latitudes where the changes in day length, and therefore the strength of the inhibitory increasing day length, are more important.

In conclusion, sexual activity of males can be induced using long days only. In addition, the stimulus provided by the males treated in this way can induce sexual activity in anovulatory females by the male effect.

Implications

The demonstration that bucks treated only with light can induce ovulations in anestrous females has at least three important practical applications for caprine productive systems in which reproductive seasonality is a major limitation. Firstly, it is a sustainable technique because it does not require application of exogenous hormones; secondly, it is an easy and nonexpensive technique to control caprine reproduction, and thirdly, it can be used in animals maintained in open sheds.

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