Induction of sexual activity of male creole goats in subtropical northern Mexico using long days and melatonin

J. A. Delgadillo*, E. Carrillo*, J. Morán*, G. Duarte*, P. Chemineau†, and B. Malpaux†

*Departamento de Ciencias Médico Veterinarias, Departamento de Producción Animal, Universidad Autónoma Agraria Antonio Narro, Carretera a Santa Fe y Periférico, Apartado Postal 940, Torreón, Coahuila, México and †INRA, Neuroendocrinologie Sexuelle, PRC-CNRS 1291, 37380 Nouzilly, France

ABSTRACT: The aim of this study was to determine whether the sexual activity of local male Creole goats in subtropical Mexico can be induced during the non-breeding season by a long-day treatment followed by insertion of two melatonin implants. The experiment was carried out in the Laguna region in the State of Coahuila, Mexico (26°N). Fourteen male goats were allocated to two balanced groups (n = 7 each) according to body and testicular weights. Males were kept together in two separate groups and fed lucerne hay for ad libitum intake and 300 g of commercial concentrate and had free access to water and mineral blocks. The control group remained in open sheds under natural photoperiod and ambient temperature conditions. The experimental group was placed in a light-proof building and exposed to 2.5 mo of long days (16 h of light/d) from November 1 to January 15. On January 16, each male received two s.c. melatonin implants and was exposed to natural photoperiodic changes in an open shed. In the control group, testicular weight exhibited seasonal variations; the highest value occurred on May 30 (146 ± 10 g). Treated males reached maximum testicular weight earlier (March 15; 147 ± 11 g), and sperm quality from January to March was higher than that observed in the control group (P < 0.05). Treatment caused an increase in LH pulse frequency (2.0 ± 0.5 vs 0.3 ± 0.2 pulse/8 h in February, 4.6 ± 1.1 vs 0.1 ± 0.1 pulse/8 h in March; experimental vs control group, respectively). In the control group, plasma testosterone remained low until mid-June and increased thereafter to remain elevated until the end of the study. In the experimental group, elevated plasma testosterone was observed from February to April and from July to November. Treating male goats in subtropical latitudes with artificial long days and melatonin can induce an intense sexual activity during the natural nonbreeding season.

Key Words: Goats, Melatonin, Photoperiod, Sexual Behavior, Subtropics

Introduction

Male goats of some populations are adapted to the environmental conditions of subtropical latitudes and exhibit large seasonal variations in sexual activity (Walkden-Brown et al., 1994a). In subtropical latitudes, nutrition is considered the limiting factor of reproductive activity and seasonal food availability is a main factor controlling timing of reproduction (Bronson and Heideman, 1994; Delgadillo and Malpaux, 1996). In support of this hypothesis, in Australian cashmere goats raised in subtropical latitudes, endocrine activity, sexual behavior, and gonadal activity are influenced by nutritional level (Walkden-Brown et al., 1994a). The sexual season begins earlier in well-nourished than in undernourished males.

In subtropical latitudes of Mexico, a marked seasonality in sexual activity has been observed in local male

1We thank the Assay Laboratory of the Station de Physiologie de la Reproduction for carrying out the radioimmunoassays; the Comité ECOS (France)-ANUIES-CONACyT-SEP (México; grant M95-B05), the Direction des Relations Internationales of the INRA, and the Scientific Attaché of the French Embassy in Mexico for making the exchanges between the two laboratories possible; Françoise Maurice-Mandon, Agnès Daveau, Sonia López, and the members of the goat reproduction team of the University Antonio Narro for their technical assistance; Dolores López for secretarial assistance; and Kirsteen Lynch of the translation department of the INRA for correcting the English manuscript.

Received September 20, 2000.
Accepted April 5, 2001.
goats maintained either in extensive conditions or constantly well-fed throughout the year. In both conditions, the breeding season lasts from May to December (Delgadillo et al., 1997, 1999). This suggests that, in such conditions, photoperiodic changes may play a more important role than expected. Indeed, seasonal variation in reproductive activity observed in natural conditions is profoundly altered when animals are subjected to alternations of 3 mo of short and 3 mo of long days. In these experimental conditions, a peak in sexual activity of both males and females occurred, as in temperate breeds, during short days (Duarte et al., 1999; Delgadillo et al., 2000).

In temperate breeds, sexual activity of males can be induced during the rest season using artificial long days followed by melatonin administration, a pineal hormone that can mimic the effects of short days (Chemineau et al., 1992). We thus tested the hypothesis that an intense sexual activity of local Creole male goats in subtropical Mexico can be induced during the natural rest season with a sequence of long days and melatonin implants.

**Materials and Methods**

**General.** The study was performed from October 1995 to November 1996 in Torreón (latitude 26°37' N), State of Coahuila, Mexico. Fourteen male Creole goats that were 1.5 yr old at the beginning of the experiment were used. The characteristics of males of this population have been reported previously (Delgadillo et al., 1999). On October 20, 1995, animals were allocated to two groups (n = 7 each) according to body and testicular weights. Males of each group were kept together and were given ad libitum access to lucerne hay (18% CP) and 300 g of commercial concentrate (14% CP) and had free access to water and mineral blocks.

**Photoperiod Treatments.** The control group remained in a 5 × 7-m open shed under natural daylength and ambient temperature throughout the experiment. Daylength varied from 13 h 41 min at the summer solstice to 10 h 19 min at the winter solstice. Animals from the experimental group were maintained in the same conditions except for the 2.5 mo (November 1, 1995, to January 15, 1996) of photoperiodic treatment when they were placed in a 6 × 5-m light-proof building and exposed to long days (16 h of light/d; lights on: 0600, lights-off: 2200). Photoperiod was regulated by an electric timer and light intensity was at least 300 lx, positioned laterally to the eyes of the animals. On January 16, all the males of the experimental group received two s.c. ear melatonin implants (18 mg each, Regulin-Mélovine, CEVA Santé Animale, Libourne, France).

**Measurements.** Body and testicular weights were measured every 2 wk throughout the experiment. Testicular weight was determined by comparative palpation with an orchidometer (Oldham et al., 1978), always performed by the same operator. From January to November 1996, sexual behavior and sperm production were assessed in all animals during the last 6 d of each month. Each of these 6-d periods was divided into two 3-d periods of daily sperm collection with 2 d of rest in between. Buck semen was collected using an artificial vagina. On each occasion, sexual activity was assessed by recording ejaculation latency and the percentage of males failing to ejaculate. Bucks had 5 min to ejaculate following exposure to an intact, estrus-induced doe (Delgadillo et al., 1991). The total number of spermatozoa per ejaculate was calculated by measuring volume and sperm concentration. Semen quality was assessed in undiluted semen by assessing the percentage of motile sperm and progressive sperm motility immediately after semen collection (Delgadillo et al., 1992). Data obtained from sexual activity and sperm production during the 1st d of each monthly collection were not used to avoid possible effects of not having collected ejaculate over a long period (Corteel, 1977). The other five monthly samples obtained from each animal were pooled for analysis.

**Blood Samplings.** All blood samples were collected by jugular venipuncture and plasma was obtained after centrifugation at 2,500 × g for 20 min and stored at −20°C until hormone concentrations were measured. Plasma LH was determined in blood samples obtained every 20 min for 8 h on February 9 and March 5 (25 and 50 d after melatonin implant insertion, respectively). Luteinizing hormone was measured by RIA as described by Pelletier et al. (1982) and validated for goats by Chemineau et al. (1982) and modified by Montgomery et al. (1985). Sensitivity (2 standard deviations from buffer control) was 0.1 ng/mL. All samples were analyzed in a single assay and the intraassay coefficient of variation was 8.6%. Plasma testosterone concentrations were determined in samples obtained weekly at 0900 throughout the experiment. Testosterone was measured by RIA as described by Garnier et al. (1978). Sensitivity was 0.1 ng/mL. The intraassay coefficient of variation was 12.3%. Melatonin levels were determined in blood samples obtained once per hour for 24 h on December 15 in order to verify the efficacy of the long-day treatment. A dim red light producing less than 3 lx was used to facilitate blood sampling at night. Melatonin was assayed by RIA as described by Fraser et al. (1983), using an antibody first raised against melatonin by Tillet et al. (1986). Sensitivity was 4 pg/mL and the intraassay coefficient of variation was 8%.

**Statistical Analyses.** Plasma concentrations of melatonin were analyzed by a two-way analysis of variance (ANOVA) with repeated measurements (group and time of day). Luteinizing hormone pulse detection was performed using the mean of the Munro software (Zaris-tow software, West Morham, Haddington, East Lothian, U.K.), according to the method of Merriam and Wachter (1982) (G parameters: G1 = 3.78; G2 = 2.20; G3 = 1.60; G4 = 1.24; G5 = 0.93; Baxter parameters: b1 = 0.07997; b2 = 0.03; b3 = 0.003). Number of pulses per 8-h period was analyzed by a two-way ANOVA with
Control of reproduction in subtropical goats

Figure 1. Pattern of mean plasma melatonin concentration during a 24-h collection period in both groups of animals. Blood samples were obtained hourly on December 15 (n = 7, control group under natural daylength [top panel]; experimental group [n = 7], 16 h of light:8 h of darkness [bottom panel]). Shaded areas represent periods of darkness.

Repeated measurements (group and month of sampling). The effect of time of the year on BW, testis weight, and plasma testosterone concentrations was analyzed by a two-way ANOVA with repeated measurements (group and time) followed by t-tests for two-by-two comparisons. Ejaculation latency, total number of spermatozoa per ejaculate, percentage of live spermatozoa, and progressive motility were also analyzed by a two-way ANOVA with repeated measures (group and time) performed on the monthly mean of each parameter followed by t-tests for two-by-two comparisons.

Results

Patterns of Melatonin and LH Secretion. Exposing the animals to long days caused a decrease in the duration of elevated melatonin concentrations by inhibiting melatonin secretion during the light phase (P < 0.001; Figure 1). The number of LH pulses was greater in the experimental group than in the control group (group effect, P < 0.001), and this difference was greater 50 d after melatonin implantation (0.1 ± 0.1 vs 4.6 ± 1.1 pulse/8 h, control vs experimental) than 25 d after it (0.3 ± 0.2 vs 2.0 ± 0.5 pulse/8 h, interaction group × time, P < 0.05; Figure 2).

Body Weight. Mean BW changed over time in both groups (effect of time, P < 0.001), but the changes occurred at different times in the two groups (group × time interaction, P < 0.001). Month-to-month differences between groups are illustrated in Figure 3a. In both groups, BW started to increase in mid-January, 10 wk after onset of long-day treatment. However, it was greater in the control than in the experimental group between the end of March and the beginning of July.

Testicular Weight. Marked changes in testicular weight were observed in both groups (effect of time, P < 0.001), but the changes occurred at different times in the two groups (group × time interaction, P < 0.001; Figure 3b). In experimental animals, testicular weight started to increase 1 wk after melatonin implant insertion and reached maximum values 4 wk later. In these treated males, testicular size was greater than in controls between late January and mid-March. In contrast, testicular weight in the control animals increased from mid-February and values were maximal at the end of May, 2.5 mo later than in the experimental group. In the control group, testicular weight was greater than in experimental animals from May to July.

Patterns of Testosterone Secretion. Large changes in testosterone secretion were observed in both groups (effect of time of experiment, P < 0.001) but the treatment modified the timing of these changes (group × time interaction; P < 0.001; Figure 3c). In control ani-

Figure 2. Number (mean ± SEM) of LH pulses detected during an 8-h period in both groups of male Creole goats on February 9 and March 5. Blood samples were collected every 20 min. The control animals (n = 7) were exposed to natural changes in daylength. The experimental animals (n = 7) were subjected to a long-day treatment between November 1 and January 15, and then to two s.c. melatonin implants.
Figure 3. Changes (mean ± SEM) in body weight (a), testicular weight (b), and testosterone secretion (c) in two groups of male Creole goats subjected either to natural changes in daylength (control, open symbols; n = 7) or to long days between November 1 and January 15 and then treated with two s. c. melatonin implants (experimental, closed symbols; n = 7). Body and testicular weight were measured twice a month. Blood samples for testosterone were taken once a week (*\(P < 0.05\); **\(P < 0.01\)).
Figure 4. Changes (mean ± SEM) in the total number of spermatozoa produced daily (a), sperm motility (b), percentage of live spermatozoa (c), and ejaculation latency (d) in two groups of male Creole goats subjected either to natural changes in daylength (control, open symbols; n = 7) or to long days between November 1 and January 15 and then treated with two s.c. melatonin implants (experimental, closed symbols; n = 7). Measurements were performed once a month during a 6-d period (see text for details) (*P < 0.05).

mals, low concentrations were observed from November to mid-June. The plasma concentrations then rose and remained elevated from July to October. Conversely, in experimental animals, two periods of high testosterone were observed, the first of which occurred from February to April and the second from mid-August until the end of study.

Sperm Production. The number of spermatozoa per ejaculate varied with time (effect of time, P < 0.001) but no effect of group or group × time interaction was observed (Figure 4a). Progressive sperm motility and percentage of live spermatozoa varied throughout the study (effect of time, P < 0.001), but these variations were much larger in the experimental group (group × time interaction, P < 0.001). In the control group, values of both variables were low in January and then increased to a maximum between May and October (Figures 4b and c). In contrast, the photoperiod and melatonin treatment increased progressive sperm motility and the percentage of live spermatozoa between January and March (difference between the two groups, P < 0.05). The values decreased thereafter but were never different from those of the control group.

Sexual Activity. There was a significant effect of time on ejaculation latency (P < 0.001) and an interaction between group and time (P < 0.001). In control animals, ejaculation latency was high between January and April, decreased from May to August, and then remained low until the end of the study. In contrast, in the experimental animals latency decreased in March and April before increasing again between May and July. Ejaculation latency was similar in the two groups between August and the end of the study (Figure 4d). In both groups, there was an effect of time of experiment on the percentage of bucks failing to ejaculate (P < 0.001). However, no interaction between group and time of experiment was found. In both groups, the percentage of bucks failing to ejaculate was high in February.
(25.7% in controls, 31.4% in experimental bucks). In contrast, the percentage was 0% between July and November in controls and between August and November in experimental bucks.

Discussion

Results demonstrate that a treatment associating long days and melatonin can induce sexual activity during the nonbreeding season in local male goats being adapted to a subtropical area in northern Mexico. Exposure to 2.5 mo of long days from November to January followed by a melatonin treatment caused an increase in LH and testosterone secretion, testicular weight, and the qualitative characteristics of the semen produced from February to April (i.e., the period when sexual activity was lowest in the control animals).

The sexual activity of males in the control group was seasonal, which confirms previous observations in male Creole goats under the same conditions (Delgadillo et al., 1999). The period of sexual quiescence, characterized by a low plasma concentration of testosterone, low, then increasing, testicular weight, and low sexual activity, was observed between January and June. In contrast, in the experimental group, the long-day treatment, applied between November 1 and January 15, followed by the insertion of the two melatonin implants caused different reproductive variables to be stimulated, which occurred earlier than in the control group. Testicular weight started to increase very soon after insertion of the melatonin implant (first significant difference on January 30) and reached a peak value in March (i.e., 2.5 mo before maximum values were observed under natural daylength in the control group). Thereafter, testicular weight decreased to reach basal values in May, when it was elevated in the control group. During this period, basal values were greater than before the stimulation, most likely as a consequence of animal growth in the meantime. The treatment-related changes in testicular weight were associated with marked changes in semen quality. Both the progressive motility of the spermatozoa and the percentage of live spermatozoa, two indexes of semen quality, were greater in treated than in control animals in January, February, and March. In contrast, quantitative sperm production was not modified by treatment, although it tended to increase in April and May. This absence of an effect is surprising because sperm output has been shown to be well correlated with testicular size in many studies (Walkden-Brown et al., 1994b; Delgadillo et al., 1995) and must be taken with caution because it may be related to the conditions of the study. Indeed, the males were maintained in a group and an increased number of mounts among them was obvious during the period of sexual activity, and probably led to an increased number of ejaculations. As a consequence, this may have led to underestimating sperm production during the tests performed over the period of sexual activity and prevented a significant effect of the treatment. In control animals, a similar phenomenon may occur during the natural sexual season. Another confounding factor may have been the frequency of sperm collection in the males. Semen was collected only six times each month in this study. It has been shown in Alpine bucks kept under natural conditions that collecting sperm twice a week leads to underestimating of sperm production during the sexual season (Delgadillo et al., 1995). The increased efficiency of spermatogenesis at this time of the year (Walkden-Brown et al., 1994b; Delgadillo et al., 1995) may cause losses of spermatozoa in urine (Lino, 1972). For these reasons, the actual sperm production during the period of sexual activity may be greater than that observed. A more intense collection frequency may have allowed observable differences between groups (Delgadillo et al., 1995).

The photoperiod treatment caused dramatic changes in LH pulsatile secretion, which was already greater in the treated than in the control group after 25 d of melatonin treatment (February 9); the difference further increased after 50 d. As a consequence, testosterone levels started to increase about 5 wk after melatonin implant insertion and remained elevated for about 2 mo. These high levels of testosterone were observed in the treated group but were minimal in the control group (February to April). In the experimental group, the subsequent diminution in testosterone concentration was probably due to refractoriness to the stimulatory short-day signal provided by melatonin implants (Almeida and Lincoln, 1984; Lincoln and Ebling, 1985). A second period of elevated testosterone secretion was observed from the middle of August (i.e., about 6 wk after the seasonal increase in testosterone concentration measured in the control group). This second period of elevated testosterone probably indicated that sexual activity was again controlled by natural daylength after melatonin implants had stopped releasing (about 100 d; Staples et al., 1991). Because melatonin concentrations were not determined in animals while they carried implants, we can only speculate that, after the exhaustion of the implants, treated animals perceived naturally increasing days from April to June. They then perceived decreasing daylength, which brought them again into a breeding condition in mid-August. This onset of activity was delayed relative to control animals, which became refractory to the long days of late spring (Gebbie et al., 1999). This increase is not associated with a change in testicular weight but it must be noted that it occurred in August, a period of the year when the testicular weight of the control animals had already decreased to basal levels while testosterone was still high. The changes in ejaculation latency were inversely related to those in testosterone level, as has been previously reported (D’Occhio and Brooks, 1976). In particular, a reduction of the ejaculation latency was observed as a consequence of the treatment in March and April when testosterone concentration was elevated.
Live weight in males of the control group displayed seasonal variations that coincided with those reported previously in males of the same breed (Delgadillo et al., 1999a) and in other breeds of sheep and goats in subtropical areas (Walkden-Brown et al., 1994a; Pérez Clariget et al., 1998). In the control group, live weight increased during the period of sexual quiescence, and thereafter stabilized without large variations during the period of sexual activity. In contrast, in the treated group, animals stopped gaining weight after March. Such variations in BW were observed in both groups despite the fact that they had ad libitum access to feed, and this occurred 2 to 3 wk after testosterone concentrations started to increase. In the treated group, BW ceased to increase during short days (melatonin treatment), whereas in the control group it ceased during long days (June). Therefore, this change in BW gain does not seem to be a direct consequence of the action of photoperiod; it is probably due to the fact that food intake was reduced as a consequence of high testosterone levels and intense sexual activity, as reported in Australian cashmere male goats (Walkden-Brown et al., 1997). Indeed, in the treated group, the absence of BW gain during the spring coincided with a high testicular weight, elevated testosterone, and an intense sexual activity. This phenomenon was observed in the control group during the natural period of reproduction.

In conclusion, our results demonstrate that a 2.5-mo long-day treatment followed by the s.c. insertion of two melatonin implants induces sexual activity in male Creole goats adapted to subtropical Mexico.

**Implications**

The present results have at least two strong practical implications. First, semen of treated animals can be used for artificial insemination with fresh semen, and this treatment allows the availability of sexually active males all year round: those induced by the photoperiodic treatment during the nonbreeding season and those naturally active during the breeding season. Second, during the nonbreeding season, treated males could be used as teasers to stimulate the sexual activity of anestrous females. Indeed, the photoperiodic treatment strongly stimulates testosterone secretion and, as a consequence, induces an intense sexual behavior necessary for obtaining a good ovulatory response in anestrous females.

**Literature Cited**


