Continuous light after a long-day treatment is equivalent to melatonin implants to stimulate testosterone secretion in Alpine male goats

J. A. Delgadillo†, L. I. Véleza and J. A. Flores

Centro de Investigación en Reproducción Caprina, Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez y Carretera a Santa Fe, C.P. 27054, Torreón, Coahuila, Mexico.

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In rams, artificial long days followed by continuous light stimulate testosterone secretion during the non-breeding season. The objective of this study was to determine whether artificial long days followed by continuous light could stimulate testosterone secretion in Alpine bucks as well as in those exposed to long days followed by a melatonin treatment. All bucks were kept in shaded open pens. Control males were exposed to natural photoperiod conditions (n = 5). Males of the two experimental groups were exposed to 2.5 months of long days from 1 December (n = 5 each). On 16 February, one group of males was exposed to 24 h of light per day until 30 June; the other group was exposed to natural variations of photoperiod and received two s.c. melatonin implants. Testicular weight was determined every 2 weeks, and the plasma testosterone concentrations once a week. In the control and the two photoperiodic-treated groups, a treatment × time interaction was detected for testicular weight and plasma testosterone concentrations (P < 0.001). In control bucks, testicular weight increased from January and peaked in June, whereas in both photoperiodic-treated groups, this variable increased from January, but peaked in April, when the values were higher than in controls (P < 0.05). In the control group, plasma testosterone concentrations remained low from January to June, whereas in both photoperiodic-treated groups, this variable remained low from January to March; thereafter, these levels increased in both photoperiodic-treated groups, and were higher than controls in April and May (P < 0.05). We conclude that continuous light after a long-day treatment stimulate testosterone secretion in Alpine male goats during the non-breeding season as well as the long days followed by a melatonin treatment. Therefore, continuous light could replace the implants of melatonin.

Keywords: goats, reproductive seasonality, photoperiod, testicular weight, subtropics

Implications

The present results show that 2.5 months of long days followed by continuous light treatment stimulate testosterone secretion in Alpine male goats during the non-breeding season, in the same way that the association of long days and melatonin. Therefore, the continuous light after the long days can replace the use of melatonin implants. The use of light is a sustainable technique to induce the sexual activity of male goats during the non-breeding season.

Introduction

The natural breeding season of male goats and rams raised at temperate latitudes lasts from September to October to February to March (Lincoln and Short, 1980; Delgadillo and Chemineau, 1992). The endocrine and reproductive seasonality is mainly controlled by variations of photoperiod: short days stimulate and long days inhibit the sexual activity (Ortavant et al., 1988; Delgadillo et al., 1991; Zarazaga et al., 2010). Thus, the manipulation of photoperiod enables to induce the sexual activity of bucks and rams during the natural sexual rest period (Chemineau et al., 1992; Leboeuf et al., 2008; Bedos et al., 2010). Indeed, exposure of bucks or rams to 2 or 3 months of long days, followed by either natural photoperiod, artificial short days or a melatonin treatment—a hormone that mimics permanent night—stimulates their endocrine and sexual activity during the non-breeding season for about 2 months (Pellicer-Rubio et al., 2008; Zarazaga et al., 2010; Delgadillo, 2011). Interestingly, in rams, long days followed by a continuous exposure to light, stimulates testosterone secretion during the non-breeding season (Malpaux and Chesneau, 2006).
In subtropical latitudes, there are few studies describing the response of Alpine bucks exposed to photoperiodic treatments. In Alpine male goats raised in subtropical Mexico at 26°N, 2.5 months of long days from 1 December followed by natural photoperiod, stimulated testosterone secretion from the end of March to the end of May, during the natural sexual rest (Delgadillo and Vélez, 2010). During these 2 months, the plasma concentrations of testosterone in the light-treated bucks were higher than control males. Whether continuous light after long days is able to stimulate testosterone secretion in Alpine bucks raised in subtropical latitudes, as reported in rams raised in temperate latitudes (Malpaux and Chesneau, 2006), is not known. Therefore, the aim of this study was to determine whether plasma concentrations of testosterone could be stimulated in Alpine male goats that received continuous light after 2.5 months of long days. Taken into account the results reported in rams exposed to long days followed by continuous light, we hypothesized that in the Alpine male goats raised at a subtropical latitude, this photoperiodic treatment would stimulate testosterone secretion of bucks during the non-breeding season, as the treatment consisting of long days followed by the insertion of the s.c. implants of melatonin.

Material and methods

The experimental procedures used in the current experiment were in accordance with the Official Mexican Rule for the technical specifications for the production, care and use of laboratory animals (NOM-062-ZOO-1999; SAGARPA, 2001).

Location and maintenance conditions of male goats

The study was performed in the Laguna region in the State of Coahuila, Mexico (Latitude, 26°23’ N and Longitude, 104°47’ W). At this latitude, day length varied from 13 h 41 min at the summer solstice to 10 h 19 min at the winter solstice. Male Alpine goats were fed alfalfa hay (18% CP) ad libitum plus 300 g/day of a commercial concentrate (14% CP; 1.7 Mcal/kg) per buck, with free access to mineral blocks and water during the whole study.

Photoperiodic treatments

A total of 15 bucks aged 3- to 4-year at the beginning of the study were used. They were divided at the beginning of the experiment into three groups (n = 5 each) balanced for testicular weight. Males of the control group remained in 3 m × 4 m shaded open pen under natural photoperiod conditions throughout the study. Males of the two experimental groups were maintained in two separate (3 × 4 m each) shaded open pens. These two groups were exposed to artificial long days between 1 December and 15 February by combining natural and artificial light. Artificial light was given from 0600 to 0800 h and from 1800 to 2200 h to extend the duration of the natural day obtaining a total of 16 h of light per day. From 0800 to 1800 h males perceived only the natural photoperiod conditions. An electronic clock controlled light on and light off and light intensity was at least 300 lx at the level of the eyes of the animals. On 16 February, one group was exposed to the natural variations of photoperiod and received two s.c. melatonin ear-implants (18 mg each; Regulin-Mélovine CEVA Santé Animale, Libourne, France). These implants released melatonin for about 10 weeks and elevated daytime concentrations to about 100 pg/ml (Staples et al., 1991). The other group received continuous light (24 h/day), and artificial light was given from 1800 to 0800 h.

Measurements

BW of each male was determined once a month. Testicular weight of each male was determined every 2 weeks by comparative palpation with an orchidometer (Oldham et al., 1978). The intensity of odor of each male was determined every 2 weeks as previously described by Walkden-Brown et al. (1997). Plasma testosterone concentrations were determined in blood samples obtained weekly from 27 January to 30 June. All blood samples were taken by jugular venipuncture. Plasma was obtained after centrifugation at 3500 × g for 25 min and stored at −20°C until hormone concentrations were measured. Radioimmunoassay measured testosterone as described by Hochereau-de Reviers et al. (1990). Sensitivity was 0.1 ng/ml, and the intra-assay CV was 7.3%.

Statistical analyses

Effects of treatments on BW, testicular weight, plasma testosterone concentrations and intensity of odor were analyzed by a two-way ANOVA with repeated measurements to test effects of photoperiod, melatonin treatment and time. When interactions between treatment and time were detected, treatment effects were examined using pairwise comparisons among treatments followed by t-test or Mann–Whitney (male odor) for 2 × 2 comparisons. Results were expressed as mean ± SEM. Analyses were computed using the SYSTAT 10 software (Evenston, IL, USA, 2000).

Results

BW

BW varied over time in males of the control and both photoperiodic-treated groups (P < 0.001). A treatment × time interaction was detected for BW, indicating that photoperiodic treatment modified the timing of these changes (P < 0.001). BW of both photoperiodic-treated males never differed between them at any time (P > 0.05), but these two groups differed from males of the control group (P < 0.001; Figure 1). In control bucks, BW increased progressively from January and peaked in May. In males from both photoperiodic-treated groups, BW also increased from January, but the highest values were registered in April, and BW decreased in May and June.

Testicular weight

Testicular weight varied over time in males of the control and both photoperiodic-treated groups (P < 0.001).
Control of reproduction of Alpine male goat

Figure 1 BW (mean ± SEM) in three groups of Alpine male goats raised at the subtropical latitude of 26° N. The control group was exposed to natural changes in day length (**). Two experimental groups were exposed to artificial long days (16 h of light per day) between 1 December and 15 February. Then, on 16 February, one group received two subcutaneous melatonin implants (○), whereas the other one was exposed to continuous light (●). BW was determined once a month.

Figure 2 Testicular weight (mean ± SEM) in three groups of Alpine male goats raised at the subtropical latitude of 26° N. The control group was exposed to natural changes in day length (**). Two experimental groups were exposed to artificial long days (16 h of light per day) between 1 December and 15 February. Then, on 16 February, one group received two subcutaneous melatonin implants (○), whereas the other one was exposed to continuous light (●). Testicular weight was determined every 2 weeks. Stars indicate that both experimental groups differed from the control group (*P < 0.05).

Figure 3 Plasma testosterone concentrations (mean ± SEM) in three groups of Alpine male goats raised at the subtropical latitude of 26° N. The control group was exposed to natural changes in day length (**). Two experimental groups were exposed to artificial long days (16 h of light per day) between 1 December and 15 February. Then, on 16 February, one group received two subcutaneous melatonin implants (○), whereas the other one was exposed to continuous light (●). Plasma testosterone concentrations were determined once a week. Stars indicate that both experimental groups differed from the control group (*P < 0.01).

A treatment x time interaction was detected for testicular weight, indicating that photoperiodic treatment modified the timing of the changes (P < 0.001). Testicular weight of both photoperiodic-treated males never differed between them at any time (P > 0.05), but these two groups differed from males of the control group (P < 0.001; Figure 2). In control bucks, testicular weight increased progressively from January and peaked in June. In males from both photoperiodic-treated groups, testicular weight also increased from January, but peaked in April, when the values were higher than in controls (P < 0.05). In June, males from the control and both photoperiodic-treated groups reached the same values (P > 0.05).

Testosterone secretion
Testosterone concentrations varied over time in males of the control and both photoperiodic-treated groups (P < 0.001). A treatment x time interaction was detected for plasma testosterone, indicating that the treatment modified the timing of these changes (P < 0.001). Testosterone concentrations of both photoperiodic-treated males never differed between them at any time (P > 0.05), but these two groups differed from males of the control group (P < 0.001; Figure 3). In the control group, plasma testosterone concentrations remained low (<5 ng/ml) during the whole experiment, from January to June. In contrast, in both photoperiodic-treated groups, testosterone concentrations increased dramatically as early as April, that is, 5 to 6 weeks after the end of the long days, and remained higher than controls in April and May (P < 0.01). Testosterone concentrations then decreased in June to reach the same levels as those of the control group (P > 0.05).

Male odor
The intensity of odor varied over time in males of the control and both photoperiodic-treated groups (P < 0.001). A treatment x time interaction was detected for males’ odor, indicating that the treatment modified the timing of these changes (P < 0.001). Males’ odor of both photoperiodic-treated males never differed between them at any time (P > 0.05), but these two groups differed from males of the control group (P < 0.001; Figure 4). In control males, odor remained low during the whole experiment, from January to June. In contrast, in both photoperiodic-treated males, odor increased dramatically as early as April, and remained higher than controls from the end of March to mid-June (P < 0.05).

Discussion
Our results indicate that the response of Alpine male goats exposed to long days followed by continuous light, is similar to the response observed in those that received the s.c. implants of melatonin. Indeed, testicular weight, testosterone secretion and the intensity of odor of males from the two photoperiodic-treated groups, increased and decreased at the same time. Interestingly, all these three variables were higher

\[\text{Body weight (kg)}\]
in both photoperiodic-treated groups than in controls in April, May and/or June, months corresponding to the natural period of sexual rest. These results validate our initial hypothesis that in Alpine male goats, continuous light after a long-day treatment stimulates testosterone secretion in a similar way as the long days followed by a melatonin treatment. Therefore, continuous light could replace the implants of melatonin.

In our study, long days followed by continuous light stimulated testosterone secretion, and high plasma concentrations of this hormone were observed in April and May, during the sexual rest period. The pattern of testosterone secretion of males exposed to continuous light was similar to that of males that received the melatonin treatment after the long days. Two elements are to be taken into account to explain the similarity between our two treated groups. First, in both light-treated groups, photoperiodic treatments caused an acute and synchronized increase of testosterone secretion in April, that is, 5 to 6 weeks after the end of exposure to long days. In ewes, it is well established that the perception of long days synchronizes the onset of the breeding season (Malpaux et al., 1989; Woodfill et al., 1994). In male goats, it was recently shown that exposure to long days also timed the onset of the breeding season, and early exposure to long days advanced the increase of plasma testosterone concentrations (Ponce et al., 2014). In our study, males from the continuous light and melatonin groups were exposed to long days from 1 December, and this exposure probably timed the increase of testosterone secretion in both groups, which occurred in April. Second, the diminution of testosterone secretion observed in June in both photoperiodic-treated groups occurred most likely as a consequence of a refractoriness to continuous light or the short-day signal provided by the melatonin implants, as reported in rams (Almeida and Lincoln, 1984; Lincoln and Ebling, 1985). Indeed, it is likely that the high plasma concentrations of testosterone observed in April in our two photoperiodic-treated groups, inhibited LH release through a negative feedback action, which in turn provoked the decrease of testosterone secretion and testicular weight, as reported in rams and bucks (Pelletier and Ortavant, 1975; Almeida and Pelletier, 1988; Delgadillo and Chemineau, 1992). This hypothesis is supported by the fact that in bucks and rams exposed to monthly alternations between long and short days, testosterone plasma concentrations were much lower than in those exposed to long and short days every 2 months or kept under natural photoperiodic conditions. Therefore, it is probable that moderate levels of testosterone secretion reduced the negative feedback action of testosterone on LH secretion, allowing them to display an intense sexual behavior and spermatogenetic activity all the year round (Delgadillo and Chemineau, 1992; Delgadillo et al., 1993; Pelletier and Almeida, 1987). All together, these data suggest that in our study, high plasma testosterone concentrations observed in bucks exposed to long days followed by continuous light or a melatonin treatment enhanced the negative feedback of testosterone, inducing the reduction of LH secretion and testicular weight in June.

In our study, testicular weight of control males increased progressively from January to June. This pattern of testicular weight observed in our study is consistent with that reported in local Mexican and Alpine bucks raised at the same subtropical latitude where the present study was performed (Delgadillo et al., 1999 and 2002; Delgadillo and Velázquez, 2010). In contrast, testicular weight in our control males increased earlier than in those raised under natural photoperiodic conditions at temperate latitudes, in which testicular weight increased from June to September (Delgadillo et al., 1991). The difference of changes in testicular weight between animals kept at subtropical and temperate latitudes may not be due to the lower amplitude of photoperiod occurring in the subtropics, but more likely to the influence of live weight changes (Walkden-Browne et al., 1994; Delgadillo et al., 1999). Indeed, in our control males, the increase of live weight, which occurred from January to May, probably induced an increase of testicular weight. This hypothesis is supported by the fact that despite the progressive increase of testicular weight from January to June observed in our control males, plasma testosterone concentrations remained low in these months corresponding to sexual rest. These observations strongly suggest that in our study, the increase of testicular weight was due to the increase of live weight and independent of LH secretion, as described in bucks and rams raised under subtropical latitudes (Walkden-Browne et al., 1994; Hötzl et al., 1997; Delgadillo et al., 1999). In males from both photoperiodic-treated groups, testicular weight also increased from January, but peaked in April, that is, 2 months earlier than the controls. In these two groups, live weight increased faster than in controls probably by a direct effect of the artificial long days. In fact, exposure to artificial long days increased live weight, probably by increasing food intake, food efficiency and secretion of growth hormone (Forbes et al., 1979; Barenton et al., 1988; Gettys et al., 1989).
Results obtained in the both photoperiodic-treated groups strongly suggest that the increase of testicular weight may have two steps to reach maximum testicular weight and testosterone secretion, as described in local bucks from subtropical Mexico (Delgadillo et al., 1999). First, the accelerated increase of live weight probably promoted the increase of testicular weight from January to April. Indeed, the increase of testicular weight was independent of LH secretion, and as consequence, low plasma testosterone concentrations were observed in these months. Second, the perception of short days by means of the melatonin treatment (Chemineau et al., 1992), or the suppression of the inhibitory effect of long days by means of continuous light (Malpaux and Chesneau, 2006), probably stimulated LH secretion, which enhanced testicular weight and testosterone secretion.

We conclude that continuous light or a melatonin treatment after long days stimulates testosterone secretion of Alpine bucks raised in subtropical latitudes. These results strongly suggest that the continuous light could replace the melatonin treatment to stimulate the sexual activity of Alpine bucks during the non-breeding season.

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Conflicts of Interest
None.

References
Delgadillo JA 2011. Environmental and social cues can be used in combination to develop sustainable breeding techniques for goat reproduction in the subtropics. Animal 5, 74–81.


Zarazaga LA, Gatica MC, Celí I, Guzmán JL and Malpau B 2010. Effect of artificial long days and/or melatonin treatment on the sexual activity of Mediterranean bucks. Small Ruminant Research 93, 110–118.