Artificial long days and daily contact with bucks induce ovarian but not oestrous activity during the non-breeding season in Mediterranean goat females

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A B S T R A C T

The aim of the present study was to determine whether a treatment of 3 mo of artificial long days and daily contact with bucks can stimulate reproductive activity during the normal seasonal anoestrous in female goats, and whether such treatment modifies the onset of the normal breeding season. Thirty-nine adult, open does were assigned to two treatments of similar mean body weight (BW) and body condition score (BCS). One treatment (LD; n = 18) was housed in a light-proof building and exposed to long days (16 h of light/d) from 17 November to 5 February, and then exposed to the natural photoperiod in an open shed. The remaining females were housed in an open shed under natural photoperiod conditions throughout the experiment (control [C]; n = 21). Plasma samples for progesterone, BW and BCS were recorded every wk. Oestrous activity was checked daily using aproned bucks. Bucks were housed close to females in a separate barn from the onset of the experiment. Ovulation rate was determined by laparoscopy 7 d after positive identification of oestrus. The interaction of treatment by time for temporal concentrations patterns of progesterone concentrations indicated that luteal activity in LD does were greater (P < 0.001) than those of C does during the natural seasonal anoestrous season. None of the C does exhibited oestrous or luteal activity during the non-breeding season; whereas, 72% of LD does exhibited luteal activity only 33% of them showed oestrous activity and during this season (P < 0.01). Differences in resumption of the oestrous or luteal activity were not observed (P > 0.05) in the subsequent breeding season between treatments. In conclusion, 3 mo of exposing does to long days and daily contact with bucks during the breeding season appears to stimulate reproductive processes that normal would not occur during the anoestrous season. However, this treatment does not induce oestrus is adequate numbers of does to be of practical value. Finally, this treatment does not modify the onset of the subsequent natural breeding season.

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1. Introduction

Seasonal changes in reproductive activity are accompanied by variations in the availability of milk or meat over the year. Out-of-breeding-season reproduction is attractive as a means of ensuring milk and meat production throughout the year in accordance with commercial needs and consumer expectations. To induce such reproductive activity, producers are moving towards practices that reduce or dispense with the use of synthetic chemicals and hormone treatments and that do not compromise animal welfare (Martin et al., 2004). Strategies such as the male effect or photoperiod treatments to control the timing of reproductive events have been proposed in order to attain the
partial reduction or elimination of exogenous hormone use (Pellicer-Rubio et al., 2007).

Since the seasonality of reproduction is under photoperiod control (Bittman et al., 1983), reproduction during seasonal anoestrus can be achieved by artificially altering photoperiods (Chemineau et al., 1986, 1988, 1999; Delgadillo et al., 2002; Zarazaga et al., 2009a, 2011). Treatments involving artificial photoperiods are based on the alternation of long and short days. Animals are first subjected to long days for 2.5–3 mo to prepare them to respond to the stimulatory effects of subsequent short days (Chemineau et al., 1986, 1988; Malpaux et al., 1989). Under field conditions, long days can easily be provided by extra illumination either indoors or outdoors, and the effects of short days can be induced with melatonin implants or natural short days. In the subtropics, photoperiodic treatment based on artificial long days alone, without the use of melatonin, has been successful in inducing sexual activity in male goats (Delgadillo et al., 2002).

Treatments that use artificial photoperiods usually require the use of additional natural methods based on the manipulation of the sociosexual structure of the group; namely, introduction of a male into a group of females that had been previously isolated from males (Chemineau, 1987; Ungerfeld et al., 2004). Isolating males from females is difficult to accomplish due to management considerations and availability of necessary equipment on farms. Véliz et al. (2006) showed that, at subtropical latitudes, previous isolation of seasonally anovulatory goats from the bucks is not a requisite to obtain stimulation of their reproductive activity by the male effect as long as novel, sexually active bucks are introduced to them.

There are very few reports on the effect of artificial long days without separation from males on the reproductive activity of females Mediterranean goats. We thus tested the hypothesis that a period of breeding activity (oestrous and luteal activity) of Mediterranean goat females that are in daily contact with bucks can be induced during the natural seasonal anoestrus using 3 mo of long days during the breeding season (short-term effect). And whether, this treatment causes a variation in the reactivation of reproductive activity in the subsequent natural breeding season (medium-term effect).

2. Materials and methods

2.1. Animals

All animal handling and experimental procedures were performed by trained personnel in strict accordance with Spanish guidelines (which conform to European Union Directive 86/609) for the protection of experimental animals (RD 1201/2005).

The study was conducted at the University of Huelva (Spain) experimental farm (latitude 37°12′N; longitude 6°54′W), which meets the requirements of the European Community Commission for Scientific Procedures Establishments (1986). The experimental animals were intact, non-pregnant, adult, Payoya female goats, all of which had kidded at least 5 months before the start of the study. These does were allocated to two treatments to achieve a similar mean body weights (BW) and body condition score (BCS) (Hervieu et al., 1991). Does in the control treatment (C; n = 21) were kept in a communal, uncovered yard with no supplementary light throughout the experiment. The remaining 18 entire does (LD) were housed in a light-proof building and exposed to artificial long days for 80 d beginning on November 17 (16 h light: 8 h dark; lights on 06:00 h: lights off 22:00 h). The duration and the moment of the light treatment was defined according to previous results that indicate the efficacy of this treatment in male goats (Delgadillo et al., 2002). Light was provided by white florescent strip lights, controlled by an electric timer. These provided approximately 200 lux at goat eye level during the light phase. A ventilator system was used for air renewal. The non-breeding season and the normal breeding season were defined according to Zarazaga et al. (2005).

On February 5, LD does were re-exposed to natural light variations and kept in the same communal yard as C does until the end of the experiment on October 26 of the following fall. During the entire experiment, animals were maintained under intensive management and were fed daily with lucerne hay, barley straw and commercial concentrate, according to INRA standards (Morand-Fehr and Sauvant, 1988), to maintain BW. The concentrate was a commercial mixture of maize (26.3%), bean (20%), oats (14.1%), cotton-seed (13.7%), pea (13.4%), lupin (7.3%), barley (0.2%), wheat (0.2%), sunflower seeds (0.2%), and a commercial mineral–vitamin complement (4.6%). All animals had free access to water and mineral blocks containing trace elements and vitamins.

2.2. Management

To avoid a male effect that might modify the reproductive activity of the does, bucks were housed close to them in a separate barn separated by a fence before the onset of the experiment. Twelve entire bucks were used for this purpose. Six were not treated and the other six received three subcutaneous melatonin implants, each containing 18 mg of the hormone (Melovine®, CEVA Salud Animal, Barcelona, Spain) after a treatment with 80 d of artificial long days beginning on November 17. The photoperiod regime was simultaneous with the LD does. The reason was to ensure that the LD does maintained contact with bucks during the artificial photoperiod regime and to enhance the reproductive behaviour of the bucks during the seasonal anoestrus (Zarazaga et al., 2010).

Oestrous activity was checked daily between November 6 until the end of the experiment by introducing aproned bucks to LD and C does. This was performed by introducing two bucks – one treated and one non-treated – simultaneously at 09:00 h for at least 30 min. This pair of bucks was then removed and a second pair introduced, until six pairs had been introduced to the females (total buck–doe contact time = 3 h). For the rest of the day bucks were isolated in their barn. Does that remained standing when a buck tried to mount, were deemed to be in oestrus. Ovulation rate was assessed by laparoscopy 7 d after oestrus.
2.3. Sampling and hormone assays

Blood samples were collected weekly from each doe by jugular venipuncture and assayed for progesterone to determine the occurrence of ovulations in the absence of oestrous behaviour and to confirm luteal activity. Immediately after collection, plasma was separated and stored at −20 °C until later use. Body weight and BCS (Hervieu et al., 1991) from each doe were assessed weekly by the same handler.

Concentration of plasma progesterone was determined by radioimmunoassay, using the technique described by Terqui and Thimonier (1974). The sensitivity of the assay was 0.125 ng/mL. The intra- and interassay CV were 8.1% and 2.1% respectively. All hormonal analyses were performed at the INRA (Nouzilly, France) hormonal analysis laboratory.

2.4. Definitions of reproductive activity

Photoperiod treatment-induced reproductive activity during the natural non-breeding season at this latitude (spring) was deemed to have begun when does exhibited their first oestrus or luteal phase. Reactivation of oestrous activity and luteal activity in the normal breeding season (autumn) was defined in a similar manner. The duration of anoestrus for each LD doe was defined as the number of days between the last detected oestrus induced by the treatment and the first detected oestrus of the following season, or the last ovulation induced by the treatment and the first ovulation of the following breeding season. The duration of anoestrus for each C doe was defined as the number of days between the last detected oestrus and the first detected oestrus of the following breeding season, while the anovulatory season was defined as the time between the last ovulation of the previous breeding season and the first ovulation of the following season (Chemineau et al., 1992).

Luteal activity was confirmed when two or more consecutive plasma samples showed progesterone concentrations above baseline (0.5 ng/mL; Zarazaga et al., 2009c) with subsequent cyclicity. For each doe, the onset of luteal activity was taken as the date of the last plasma progesterone concentration below baseline that was followed by the first extended cyclic pattern. Cessation of luteal activity was considered to have occurred when three or more consecutive plasma samples had concentrations below baseline. The date of the first plasma progesterone concentration below baseline at the completion of the last extended cyclic progesterone pattern was taken as the end of luteal activity.

Variables associated with establishment of cyclicity included: (i) oestrous activity (oestrous behaviour accompanied by an increase in plasma progesterone concentrations above 0.5 ng/mL in at least two consecutive samples), (ii) luteal activity (with an increase in plasma progesterone concentrations above 0.5 ng/mL in at least two consecutive samples but not preceded by detected oestrous behaviour), and (iii) ovulation rate of each detected cycle of does that exhibited oestrus.

![Graph showing changes in body weight (BW) and body condition score (BCS) over time](image)

**Fig. 1.** Weekly means for body weight (BW) and body condition score (BCS) in Mediterranean does subjected to natural changes in day length (C treatment, •) or after exposure to 16 h photoperiod from November 17 to February 5 and then exposed to the natural photoperiod (LD treatment, •). The shaded area indicates the duration of the exposure to long days. Bars associated with means represent SE for each mean. Interaction of treatment by time (P<0.001) for BW but not for BCS.

2.5. Statistical analysis

All calculations were made using SPSS software (SPSS, 2006). The effect of the treatment and time on BW, BCS and progesterone was analysed using repeated measures analysis with time (week) and treatments (LD and C treatment) as sources of variation. ANOVA was used to examine the effect of treatment on the date of the first detected oestrus, and the first luteal activity in the normal breeding season. The effect of treatment on ovulation rate of the first detected oestrus of the natural breeding season was compared using the Mann–Whitney U-test. The percentage luteal and oestrous activity detected in the two treatments during the natural breeding season was compared using the χ² test.

3. Results

3.1. Body weight and body condition score

There was a treatment by time interaction effect (P<0.001) for changes in BW. Body weight increased slightly in LD does during the artificial long-day period; whereas, C does lost (P<0.01) BW during this same period (Fig. 1). Thereafter, LD does experienced losses of BW (−56.7±14.9 g/d) whereas the C does experienced gains in BW (24.3±15.2 g/d) (P<0.001). Finally, during the reactivation of reproductive activity in the normal breeding
season (September) until the end of the experiment, the C does experienced losses of BW (−38.9 ± 12.8 g/d) whereas the LD does maintained their BW (−2.1 ± 8.2 g/d) (P < 0.05).

An effect of time (P < 0.001) was observed on BCS (Fig. 1). There was a slight decrease in both treatments one month after the end of the photoperiod protocol and a slight increase until the end of the experiment but neither treatment nor the interaction of treatment by time had any influence.

3.2. Plasma progesterone concentrations

Fig. 2 shows the changes in plasma progesterone concentrations and as a consequence the luteal activity. There was a treatment by time interaction effect (P < 0.001) for temporal concentration patterns. Progesterone concentration in LD does decrease earlier (13 January ± 5.4 d) than those in C does (21 February ± 4.6 d) and remained low in does of both treatments after February until mid-April. The LD does showed higher progesterone concentrations during the seasonal anoestrus than C does. Progesterone concentrations rose in LD does 60.5 ± 4.4 d after the end of the artificial long days, and remained high until late May. Thereafter it decreased to baseline concentrations in LD does, however, in C does, remained at baseline concentrations during this time.

On the whole, more LD does (72%) (Fig. 3) exhibited luteal activity (P < 0.05) during a 50.6 ± 6.6-d period from early-April to late-May, whereas, none of the C does showed luteal activity during this time. However, the incidence of ovulation without oestrus was 54% for LD does that exhibited luteal activity. The incidence of short cycles (less than 16 d) during this period was 14.3% for the LD does.

Differences in the onset of the luteal activity, as measured by plasma concentrations of progesterone, were not observed between both treatments in the subsequent breeding season (September 18 ± 3.3, P > 0.05) when LD treated does were placed under natural photoperiod. Luteal quietude between last luteal phase at the end of the natural breeding season and the first of the next breeding season was longer (P < 0.001) in C (214.1 ± 8.1 d) than LD (107.9 ± 5.8 d) does. The onset of the normal breeding season was characterised by a very high incidence of short cycles in both treatments (70% vs 40% for LD and C does, respectively) and silent ovulations (36% vs 44% for LD and C does, respectively).

3.3. Oestrus activity and ovulation rate

Interval from initiation of artificial long days to cessation of behavioural oestrus was shorter (P < 0.001) for LD does (11 January ± 5.7 d) than for C does (19 February ± 4.6 d). During natural seasonal anoestrus, no oestrus activity was observed in C does and the mean date for the first detected oestrus of the LD does was April 12 ± 8.9 d. On the whole, 33% of LD does showed oestrus during the natural anoestrous season and was higher (P < 0.05) than in C does (Fig. 3).

Differences in resumption of the rest oestrus activity (220.9 ± 8.6 d) (P < 0.05) and in the onset of the subsequent breeding season (September 27 ± 3.3 d) between treatments (P < 0.05) were not observed.

Ovulation rate for the first detected oestrus during the natural anoestrous season in LD does was 1.67 ± 0.2 corpora lutea (none female of the C group showed oestrus). Treatment had no effect on this variable for the first detected oestrus of the subsequent natural breeding season (1.67 ± 0.1 corpora lutea) (P > 0.05).

4. Discussion

The results of the present study demonstrate that artificially increasing the photoperiod for 3 mo during the breeding season to 16 h/d reduced the length of the breeding season in LD does compared to that of control does and caused early onset of seasonal anoestrus. Thereafter, this treatment caused a reactivation of progesterone concentration in these does during the natural seasonal anoestrus at Spanish latitudes. This indicates that photoperiod could be a major factor that regulates the occurrence and cessation of ovarian activity of Payoya does. The natural photoperiod following a period of artificially increased photoperiod was interpreted by the animals as one of short days despite it being a time of increasing day length. Two hypotheses, perhaps not mutually exclusive, might explain this. Firstly, in sheep, it is well documented that seasonal variations in reproductive activity are the expression of an endogenous rhythm and that the perception of long days in the spring is a critical synchronizing signal of the rhythm involved in timing the onset of the breeding season (Woodfill et al., 1994; Malpaux et al., 2001). Although this mechanism has not been demonstrated in goats, it might be assumed to act in the same way. Therefore, the exposure of the animals to long days from November to February 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 February (about 10 h 30 min of light/d) could have caused an acute stimulation of reproductive activity. However, it must be noted that, after this initial stimulation, day length increased progressively and pro-
vided a potentially inhibitory signal (Malpau et al., 1989). Nevertheless, the induction of ovulatory cycle during the natural anoestrous season obtained by the phase shift in the endogenous rhythm due to the shortened photoperiod is not cancelled out by this inhibitory signal. This observation, made under Mediterranean conditions in which the change in day length between the winter solstice and the spring equinox is about 2 h 34 min, is similar to that seen under subtropical conditions where the change is only about 1 h 40 min (Delgadillo et al., 2002). It would be interesting to determine whether this observation holds at higher latitudes where the change in day length, and therefore strength of the inhibitory increasing day length, is greater.

Three months of long photoperiod during the natural breeding season induced luteal activity in does during the natural anoestrous season but did not appear to alter the occurrence of oestrus in these does. These results differ from those of Gatica et al. (2007) who used a melanotin implant at the end of the 3-mo increase in photoperiod during the natural breeding season and reported that most does showed oestrus and ovulated in the natural anoestrous season. The results of the present study in relation to other studies may indicate that, in Mediterranean goats, 3 mo of long photoperiod exposure during the breeding season need to be associated with other practices as the male effect, or melanotin insertion at the end of the photoperiod regime, to increase the occurrence of oestrus.

Moreover, in the present work none of the C does showed luteal or oestrous activity during the anoestrous season even they were in daily contact with sexually active does and bucks. However, in a study by Walkden-Brown et al. (1993), 87% of goats responded to the male effect when other oestrous does were present compared to 72% when such females were absent. In fact, the presence of only a few oestrous ewes or goats may induce sexual activity in the remaining females of a flock, even in the absence of any male (Bouillon et al., 1982; Restall et al., 1995). Similarly, Vélez et al. (2006) showed that, at subtropical latitudes, previous isolation of seasonally anoestrous goats from the bucks is not a requisite to obtain stimulation of their reproductive activity by the male effect as long as novel, sexually active bucks are introduced to them.

In addition, a very high incidence of irregular reproductive activity (silent ovulation, short cycles) was observed in the present study. This has been described as a regular occurrence at the onset of the breeding season before the start of normal reproductive activity. The reason for this may be that progesterone may increase the sensitivity to LH after a period of anoestrous (Legan et al., 1985).

The occurrence of oestrus and luteal activity in the subsequent normal breeding season did not differ between LD and C does. These results differ from those obtained by our group using photoperiod-melanotin-treated bucks or bucks treated with melatonin at the spring equinox (Zarazaga et al., 2010) or does treated with melatonin at the spring equinox (Zarazaga et al., 2009a) or photoperiod-melanotin-treated does (Gatica et al., 2007). In all of these studies, the onset of the normal breeding season for bucks and does was delayed. The disparity between those studies and the present study might be explained because the time of reproductive onset in autumn depends on the time of exposure to long days after the winter solstice.
(Malpaux et al., 1989). The reason for the previous results probably lies in the exhaustion of the melatonin implant, which delayed perception of the natural long days. However, in the present experiment, no melatonin treatment was performed; consequently, the moment of the year that determined the onset of the normal breeding season was not modified.

Exposing does artificially to long days during the natural breeding season did not appear to influence ovulation rate early in the next normal breeding season. This observation may mean that follicular wave dynamics of does may not be altered by inducing luteal activity during the natural anoestrus season of Mediterranean goats. As far as we know this is the first report of this observation in goats.

The reproductive changes observed were linked to changes of BW observed in both treatments. Body weight increased during periods of reproductive rest and losses of BW were observed during the periods of increased reproductive activity, despite the fact that the animals were fed to maintain BW. Our group (Zarazaga et al., 2005, 2009a,b, 2010) has previously reported such variations in BW in this species. However, the changes in body weight differed between LD and C does. Differences in food intake could be the origin of these changes (Argo et al., 1999). One reason for this is likely to be that the higher steroid concentrations recorded during the breeding periods directly inhibit voluntary feed intake (Newman et al., 1992; Walkden-Brown et al., 1997) and changing metabolic status during the year (Walkden-Brown et al., 1997). Another possibility is that photoperiod has a direct effect on body weight, and it is known that weight gain is stimulated by long days and inhibited by short days (Barenton et al., 1988).

In conclusion, the results of this study support the hypothesis that artificially increasing the photoperiod for three months during the natural breeding season and daily contact with bucks can induce the occurrence of endocrine reproductive activity during the natural anoestrous season in Mediterranean does without affecting the onset of the next natural breeding season. However, very few do respond in terms of oestrous activity, indicating that by itself such stimulation is insufficient to induce a sufficient number of does to exhibit oestrous and be used as reproductive management strategy for out-of-season breeding in Mediterranean goats. Further study is required to determine what other treatments might be associated with this photoperiod treatment in order to obtain adequate oestrous activity.

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