Animal Reproduction Science 202 (2019) 58–64

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Photoperiod-treated bucks are equal to melatonin-treated bucks for inducing reproductive behaviour and physiological functions via the "male effect" in Mediterranean goats

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ARTICLE INFO

Keywords:
Male effect
Seasonality
Photoperiod
Melatonin

ABSTRACT

The aim of this study was to examine whether photoperiod-treated bucks have the same capacity as melatonin-treated bucks to induce reproductive responses in female goats during the spring. On 10 April, 38 anoestrous does were placed with: 1) photoperiod-treated bucks (additional light hours for 83 days from the end of the previous November; PHOTO; $n = 12$); 2) bucks treated with exogenous melatonin at the beginning of March (MEL; $n = 13$); and 3) bucks that received no treatments (CONTROL; $n = 13$). The bucks' sexual behaviour was assessed for 10 days, and doe oestrous behaviour was recorded for the next 32 days by checking for harness marks. Ovulation was confirmed from plasma progesterone concentration (measured twice per week) and ovulation rate was assessed by transrectal ultrasonography. Fecundity, fertility, prolificacy and productivity were also determined. The percentage of does in the PHOTO, MEL and CONTROL group: 1) having ovulations was 92%, 100% and 38% respectively; 2) expressing behavioural oestrous associated with ovulation was 92%, 100% and 31%; and 3) that became pregnant was 75%, 69% and 23%, respectively. The kids produced per doe were 1.08 ± 0.23, 1.15 ± 0.25 and 0.31 ± 0.17 for the PHOTO, MEL, and CONTROL groups, respectively with there being no differences between the PHOTO and MEL groups, however, there was a difference ($P < 0.05$) between PHOTO/MEL and CONTROL groups. Thus, imposing a stimulatory lighting regimen during the preceding winter period is as efficient as using subcutaneous melatonin implants in stimulating reproductive behaviours and physiological functions of bucks.

1. Introduction

Most breeds of goats from temperate latitudes have seasonal variations in sexual activity (i.e., reproductive behaviours and physiological functions) (Chemineau et al., 1992; Zarazaga et al., 2005; Gallego-Calvo et al., 2014). This is regulated by an

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https://doi.org/10.1016/j.anireprosci.2019.01.008
Received 12 September 2018; Received in revised form 18 January 2019; Accepted 28 January 2019
Available online 30 January 2019
0378-4320/

endogenous rhythm that is synchronised primarily by the photoperiod. Daylight stimuli are received by the retina and transmitted to the pineal gland, which secretes basal amounts of melatonin because of this stimulus. Melatonin, therefore, is the hormone that regulates the endocrine reproductive axis (Gerlach and Aurichr, 2000). Melatonin is synthesized and secreted in a daily rhythmic pattern: with relatively greater concentrations being secreted during the dark phase of a light-dark cycle, and basal concentrations being secreted during the light phase (Reiter, 1991; Zawilska and Nowak, 1999). Decreasing length of the photoperiod results in a stimulation of reproductive activity in bucks and does, while increasing the photoperiod has the opposite effect (Chemineau et al., 1988; Delgadillo and Chemineau, 1992; Duarte et al., 2008, 2010; Zarazaga et al., 2011a).

In does, reproductive seasonality can be modified by sociosexual interactions with bucks. The introduction of a buck with seasonally anovulatory does can induce ovulations. This is known as the "male effect", and it has been extensively studied in ewes and goats (for a review see Delgadillo et al., 2009). On the extensive and semi-extensive goat farms of the Mediterranean region, using the "male effect" to induce reproductive functions in does is a common reproductive management practice. The extent to which sexual behaviour is expressed by the bucks determines the proportion of does having ovulations (Flores et al., 2000; Delgadillo et al., 2001; Zarazaga et al., 2010; Delgadillo et al., 2014; Chasles et al., 2016). With Mediterranean conditions, sexual activity of bucks is naturally reduced from February to September, and the proportion of females having ovulations in response to placement of bucks with does is markedly less during this period (Zarazaga et al., 2005, 2009; Gallego-Calvo et al., 2014, 2015). To overcome this problem, photoperiod and/or melatonin treatments can be used. Exogenous melatonin from continuous slow-release implants inserted subcutaneously around the spring equinox can be administered to advance the onset of the breeding season in bucks by hormonally mimicking short days, with the outcome of this treatment being increases in plasma testosterone, ejaculate volume, mean semen concentration and number of sperm cells per ejaculate (Zarazaga et al., 2010). Alternatively, if bucks are maintained in open barns, long day photoperiods can easily be imposed by extra illumination for 2.5 to 3 months during the winter (Chemineau et al., 1986, 1988; Malpaux et al., 1989). The effect of short photoperiods can then be induced by simply exposing bucks to natural springtime day length conditions. This photoperiod is shorter than the previous artificial photoperiod, and, therefore, stimulates enhancement of sexual activity. Such treatments have been successfully used to enhance sexual activity in male goats (Delgadillo et al., 2002; Zarazaga et al., 2017a, b; Gallego-Calvo et al., 2018; Zarazaga et al., 2018). Nonetheless, while melatonin and artificial photoperiod treatments have been extensively studied as a means of stimulating buck reproductive activity at Mediterranean latitudes (Zarazaga et al., 2010, 2017a, b) these two approaches have never been compared in terms of effectiveness for enhancement of buck reproductive behaviours and physiological functions.

The aim of the present study was to compare with an untreated control group the effectiveness of photoperiod manipulations and melatonin treatments on the response of does to the "male effect".

2. Material and methods

2.1. Study conditions

All animals were managed in pens with an uncovered area and a covered area. All procedures were performed by trained personnel in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013), and in agreement with European Union Directive 86/609. The procedures of the present experiment were evaluated by the qualified organisation of the ethical committee for animal experimentation (CEEA-OH) from the University of Granada and authorised with the reference number 297-CEEA-OH-2018. The study was conducted at the experimental farm of the University of Huelva (latitude 37° 20′N and longitude 6° 54′ W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

2.2. Treatment of bucks to induce the "male effect"

There were three groups ($n = 4$ each) of sexually experienced Blanca Andaluza bucks used to induce the "male effect", which were 5 years-old at the beginning of study. On 24 November, a group of males housed in open barns were exposed to long photoperiods (16 h light: 8 h dark; lights on 0600: lights off 2200) for 83 days (photoperiod-treated bucks). The photoperiod was regulated by an electric timer that controlled white fluorescent strip lights providing approximately 200 lx at the level of the eyes of bucks. At the end of the photoperiod treatment (i.e., on 15 February of the following year), the bucks continued to be maintained in natural photoperiod conditions. On the 3 March, another group of bucks was administered two subcutaneous melatonin implants containing 18 mg of melatonin (Melovine®; CEVA Salud Animal, Barcelona, Spain) at the base of the left ear (melatonin implant-treated bucks). The other group of bucks was exposed to natural photoperiod throughout the experiment (untreated bucks; i.e., CONTROL). Bucks of each group were housed in separate pens.

2.3. Preparation of does

There were three groups of does utilized in this study with does of each group being placed with bucks of one of the three groups (photoperiodic treated; melatonin implant; control). The females were multiparous ($n = 38$), seasonally anovulatory Blanca Andaluza goats, which were 4 or 5 years old at the beginning of study. Parturitions occurred in these females between September and October. Does were assigned to one of the three groups with stratification of assignments based on body weight (BW) and body condition (BC; as determined by the procedures of Hervieu et al., 1991) with does being placed in the pen with 1) photoperiod-treated bucks (PHOTO; $n = 12$; BW: 43.3 kg ± 1.1; BC: 2.94 ± 0.06); 2) melatonin implant-treated bucks (MEL; $n = 13$; BW:

43.2 kg ± 1.0; BC: 2.90 ± 0.07); or 3) untreated bucks (CONTROL; n = 13; BW: 43.0 kg ± 0.8; BC: 2.90 ± 0.07). The does were penned together until the time of placement with the bucks when the three groups were established. At this time, animals were housed in open barns completely isolated from those animals in the other treatment groups. All animals were fed lucerne hay, barley straw (*ad libitum*) and commercial concentrate daily to maintain body weight based on diets calculated using INRA requirements for goats that had a body weight of 45 kg (Morand-Fehr and Sauvant, 1988). All animals had free access to water and mineral blocks containing trace elements and vitamins.

2.4. "Male effect"

On 10 April, 54 days after the end of the photoperiod treatment, or 38 days after inserting the melatonin implant, four of the eight treated bucks (as described at the section 2.2) were selected for use in the study. During the previous week bucks had all been exposed for 5 min on 1 day to does in oestrus (not the experimental does), and their sexual behaviour was assessed by observing genital sniffing, nudging and mounting attempts. Two bucks of the PHOTO and MEL groups, expressing similar aspects of sexual behaviour were selected and placed with does of the two groups (PHOTO and MEL, respectively). Two bucks chosen at random from the group of untreated bucks were assigned to the CONTROL group of females. Bucks were equipping with marking harnesses and then placed with the experimental does and kept with them for the following 32 days (until 15 May).

2.5. Variables recorded for the does

2.5.1. Detection of oestrous behaviour, ovulation and ovulation rate

Oestrous behaviour was recorded every day by direct visual observation of the marks from the marking harnesses (Walkden-Brown et al., 1993). The interval between the time of buck placement with does and the first detected oestrous behaviour was calculated for each doe.

To monitor the ovulatory cycles of does before placement with bucks (Day 0; 10 April), blood samples were collected once per week for three consecutive weeks and the plasma progesterone concentration was determined. Blood was collected by jugular venipuncture in tubes containing 10 μl heparin and plasma was obtained by centrifugation at 3500 × *g* for 30 min, and stored at −20 °C until analysis. Females with plasma progesterone concentrations ≤1.0 ng/ml in all samples were considered to be anoestrus (Chemineau et al., 1992). Plasma progesterone concentration was determined twice per week to monitor the ovulatory response after male introduction. Does with plasma progesterone concentrations ≥1.0 ng/ml in at least two consecutive samples were deemed to have had ovulations and to have developed a corpus luteum of normal functional duration (Chemineau et al., 1992). The date of detection of this ovulation was defined as that when the first sample with progesterone concentrations of ≥1.0 ng/mL. Ovulations without detection of behavioural oestrus were deemed to have occurred when there was an increase in plasma progesterone ≥1.0 ng/ml that was detected in at least one sample, but was not preceded by oestrous behaviour. The percentages of does expressing behavioural oestrus with or without ovulation, as well as those having an ovulation without detection of behavioural oestrus were inferred based on the profiles for plasma progesterone concentrations.

The occurrence of ovulation and the ovulation rate were assessed by the number of corpora lutea observed in each female by transrectal ultrasonography conducted 6–8 days after the detection of oestrus (Simoes et al., 2005). The procedure was performed using an Aloka SSD-500 (Ecotron, Madrid, Spain) apparatus connected to a 7.5 MHz linear probe.

2.5.2. Plasma samples and hormone analysis

Plasma progesterone was determined using an enzyme-linked immunoassay kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer's instructions (Andueza et al., 2014). The sensitivity of the assay was 0.2 ng/mL. Intra- and inter-assay coefficients of variation for sample pools of 0.5 and 1 ng/ml were 5.5%, 7.5%, and 7.8% and 7.7%, respectively.

2.5.3. Fecundity, fertility, prolificacy and productivity

Fecundity (percentage of pregnant does/does mounted by the males) was determined using transrectal ultrasonography on day 45 after oestrus was detected (Schrick et al., 1993). Fertility (percentage of does kidding per doe that mated), prolificacy (number of kids born per female kidding) and productivity (number of kids born per female that mated) were also determined (Caravaca et al., 1999).

2.6. Buck plasma testosterone and sexual behaviour

Blood for the determination of plasma testosterone was obtained by jugular venipuncture and using vacuum tubes containing 10 μl of heparin. Blood samples were collected weekly at 09:00 h from the onset of the experiment (31 October until 15 May). Plasma was processed as previously described in this manuscript. Testosterone concentrations were determined using a commercial enzyme-linked immunoassay kit (Demeditec Diagnostics, Kiel-Wellsee, Germany). The sensitivity of the assay was 0.1 ng/mL. Intra- and inter-assay coefficients of variation for sample pools of 0.2 and 6.0 ng/ml were 3.2%, 3.0%, and 5.5% and 5.2%, respectively.

The sexual behaviour of the bucks was also observed for 30 min (from 8:00 to 8:30) on Days 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 post-placement of bucks with does (Day 0 = the day of introduction). Genital sniffing (buck sniffing the ano-genital area of does); licking (buck licking flanks of does); nudging (buck kicking doe); sneezing sounds (bucks emitting a sneezing sound); mounting attempts (buck attempting to mount does without intromission) and mounting with intromission (bucks mounting the does with intromission) were all recorded. The sexual behaviour of all bucks was monitored using a video recording system, thus avoiding human interaction with the animals.

2.7. Statistical analyses

Data are presented as means ± standard error. The weekly values for testosterone concentrations were analysed using an ANOVA with time as a repeated measure and the experimental treatment of the males as the main factor. The Tukey test was used to detect weekly differences between groups.

The variables expressed as percentages - does with ovulations, those expressing oestrous behaviour and having ovulations, fecundity and fertility - were analysed using the Fisher-Freeman-Halton exact probability test for multiple group comparisons, and the Fisher exact probability test for two-group comparisons as required. Ovulation rates and prolificacy were compared using the Mann-Whitney U test. Productivity and the dates of ovulation, and ovulation with oestrous behaviour were compared using a one-way ANOVA with the male treatment as a fixed effect.

The percentage of genital sniffs, licks, nudges, sneezing sounds, mounting attempts, and mounting with intromission were calculated for each group and analysed using the Fisher exact probability test for two-group comparisons. Significance was set at $P < 0.05$. All calculations were performed using the STATA14 software package (StataCorp, 2015).

3. Results

3.1. Testosterone concentrations and sexual interactions with bucks

Time had a marked effect on the plasma testosterone concentration ($P < 0.01$), as did the interaction time x buck treatment ($P < 0.01$). The bucks of the PHOTO and MEL groups had greater testosterone concentrations than the buck of the control group from 27 March until 24 May (Fig. 1) - precisely when bucks were placed with does for induction of the "male effect".

The bucks of the MEL and PHOTO groups performed more genital sniffing, licking and nudging than bucks of the CONTROL group ($P < 0.01$; Fig. 2). The bucks of the MEL group made more sneezing sounds than the bucks of the CONTROL group ($P < 0.01$; Fig. 2) and the bucks of the PHOTO group ($P < 0.01$). The bucks of the MEL group also performed more genital sniffing and licking than the bucks of the PHOTO group ($P < 0.01$; Fig. 2), but the bucks of the PHOTO group nudged the does more often than did the bucks of the MEL group ($P < 0.01$). There were no differences between the bucks of the PHOTO and MEL groups in terms of mounting attempts, or mounting with intromission ($P > 0.05$; Fig. 2).

3.2. Doe reproductive response

Compared to the does of the CONTROL group, more of the does of the PHOTO and MEL groups expressed oestrous behaviour and had ovulations ($P < 0.05$; Table 1) and there were no differences between the responses of does in the PHOTO and MEL groups. Similarly, there were no differences in fertility and productivity between does of the PHOTO and MEL group, however, the values for

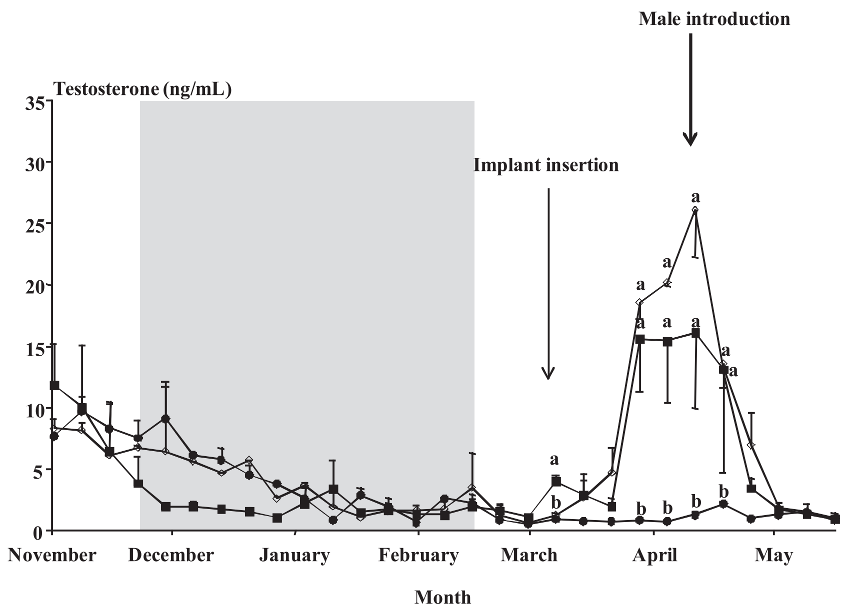

Fig. 1. Plasma testosterone concentration (ng/mL) of males submitted to a photoperiodic treatment, i.e., long days for 3 months between November and February (■, $n = 2$); males implanted with exogenous melatonin from 3 March (◊, $n = 2$) and untreated males (●, $n = 2$); Values with different letters (a,b) differ ($P < 0.05$).

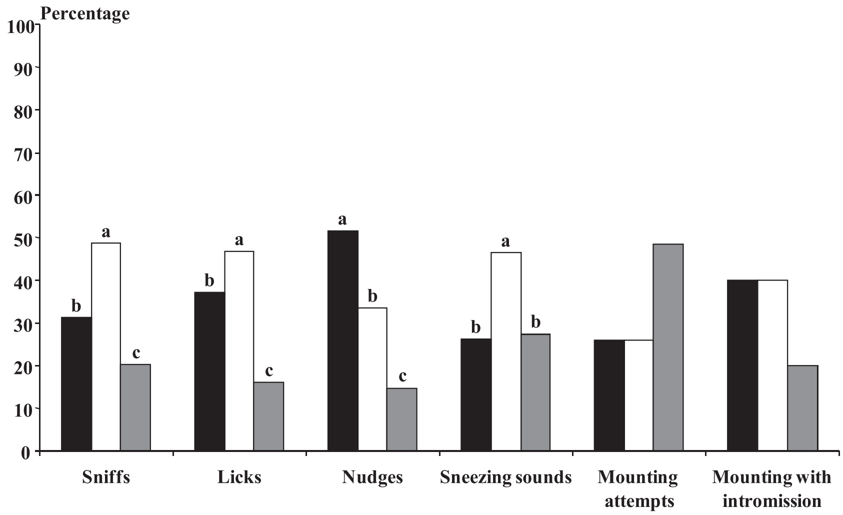

Fig. 2. Distribution of each type of behaviour after imposing the "male effect", observed in the three groups of males, expressed as a percentage of the total number of behaviour characteristics; Sexual behaviour was recorded for 30 min daily during the first 9 d post-placement of bucks with the does; Male effect was imposed using photoperiod-treated males, i.e., long photoperiods for 3 months between November and February (■, $n = 2$), using males implanted with exogenous melatonin on 3 March (□, $n = 2$), and untreated males (■, $n = 2$); Different letters differ ($P < 0.01$).
Sniffs (when the buck sniffing the ano−genital area of the doe).
Licks (when the buck licks the flanks of the doe).
Nudges (when the buck kicked the doe).
Sneezing sounds (sounds emitted by the bucks).
Mounting attempts (when the buck attempts to mount the doe without intromission).
Mounting with intromission (when the bucks mounts the doe with intromission).

Table 1
Reproductive response of does submitted to the male effect in Spring using males submitted to a photoperiodic treatment, i.e., long days for three months between November and February (PHOTO); males implanted with exogenous melatonin from March 3rd (MEL) and untreated males (CONTROL).

	PHOTO ($n = 12$)	MEL ($n = 13$)	CONTROL ($n = 13$)
Females ovulating (%)	92[a]	100[a]	38[b]
Interval introduction of male- normal ovulation	15.64 ± 0.36	15.92 ± 0.94	14.80 ± 1.11
Females in oestrus and ovulating (%)	92[a]	100[a]	31[b]
Interval introduction of male- oestrus	9.5 ± 0.8	10.6 ± 1.1	9.8 ± 1.5
Ovulation rate	1.40 ± 0.22	1.25 ± 0.16	1.25 ± 0.25
Fecundity (%)	91	77	100
Fertility (%)	75[a]	69[a]	23[b]
Prolificacy	1.44 ± 0.18	1.67 ± 0.17	1.33 ± 0.18
Productivity	1.08 ± 0.23[a]	1.15 ± 0.25[a]	0.31 ± 0.17[b]

Different letters in the same row reflect significant differences among the groups ($P < 0.05$).

fertility and productivity were greater in these two groups than the CONTROL group ($P < 0.05$; Table 1).

The interval from placement of bucks with does to first ovulation was shorter than the interval from the time of buck placement with does to detection of oestrus (10.04 ± 0.62 days compared with 15.62 ± 0.47 days; $P < 0.001$), with no differences between groups ($P > 0.05$).

4. Discussion

The results of the present study indicate, at Mediterranean latitudes, treatment with artificial long days for 3 months between November and February increases the testosterone concentration to the same extent as melatonin implant insertion (with both treatments leading to greater testosterone concentrations). Bucks treated in either method interacted more frequently with does than did bucks of the control group, leading to greater doe reproductive performance (with no significant differences between photoperiod- and melatonin-treated bucks). Photoperiod-treated bucks, therefore, when placed with does induce reproductive responses in does to an equal extent as melatonin-treated bucks, and could be used to induce the "male effect" on farms where hormone treatments are prohibited.

The photoperiod and melatonin treatments both stimulated testosterone secretion during what would normally be the sexually inactive period for goats at Mediterranean latitudes. This result is similar to that of previous studies with bucks of the Payoya breed (Zarazaga et al., 2010), however, in the previous study there was no adequate comparison of the effect of photoperiod and melatonin treatments that could be made.

In goats, a switch between long and short photoperiods is needed to stimulate reproductive behaviour and physiological functions (Delgadillo and Chemineau, 1992; Zarazaga et al., 2010, 2011a,b,c). In the present study, in both groups of treated bucks there was a switching between long and short photoperiods that resulted in enhancement of reproductive behaviour and physiological functions to a similar extent. Seasonal variations in reproductive activity are the expression of an endogenous rhythm, and the perception of long photoperiods in the spring is an important factor for synchronizing the signals involved in timing the onset of the breeding season (Woodfill et al., 1994; Malpaux et al., 2001). Thus in the photoperiod-treated bucks, exposure to long photoperiods (16 h of light/d) in November to February resulted in an induction of reproductive activity. The shift from these artificially long photoperiods to the naturally shorter photoperiods in February would, therefore, induce an acute stimulation of reproductive activity. It should be noted that from January to March, length of the photoperiod increases progressively and could provide a potentially inhibitory signal (Malpaux et al., 1989). This inhibitory signal, however, did not suppress the stimulatory effect obtained by the phase shifting of the endogenous rhythm and the initial stimulation caused by the decrease in photoperiod. Similarly, in the melatonin-treated bucks, a sexual activity-inducing short day signal is received because of the melatonin treatment following a preceding period of comparatively long days (even though these days are in fact the shortest of the year).

The present findings clearly indicate that both the melatonin and photoperiod treatments render the bucks fully capable of inducing reproductive activity in does during their anoestrous period. Of the does placed with the bucks of the control group, only 31% expressed oestrus, while almost all those in contact with either type of treated bucks had ovulations and expressed oestrous behaviour. This confirms results suggesting that buck reproductive state is a key factor for induction of reproductive responses in a large percentage of does. Doe response to bucks is more profound when sexually active males are used (Flores et al., 2000; Delgadillo et al., 2002; Véliz et al., 2002). Furthermore, the fertility of does mated with the treated males was 72%, suggesting that sperm production in the treated bucks was highly acceptable, both qualitatively and quantitatively. Productivity was also much greater (+80 kids per 100 goats mated) among the does of the PHOTO and MEL groups as compared with those of the CONTROL group. Results of research with Murciano-Granadina goats (Zarazaga et al., 2012) comparing females mated with bucks treated with or not treated with melatonin indicates the increase in the productivity was less than that observed in the present experiment (+32 kids per 100 goats mated). This result probably occurred because the does that mated with the bucks of the CONTROL group in the previous study had greater productivity than those in the present experiment.

The interval from 'buck placement with does to time of detection of oestrous behaviour' was shorter than that for 'buck placement with does to the first increase in progesterone concentration' and the values for these variables were similar for bucks of the two treated and control groups. The main reason for this is likely that the procedure used to estimate the interval from 'buck placement with does to the first increase in progesterone concentration' involved checking for progesterone concentrations of ≥ 1 ng/mL. This only occurs when the corpora lutea are fully functional, several days after oestrous behaviour is expressed (Zarazaga et al., 1996). Accordingly, the does in the present study had a typical response to the “male effect” with a short ovarian cycle unaccompanied by behavioural oestrus, and the first oestrus accompanied by ovulation occurred about 10 days after placement of does with bucks (Chemineau, 1983). There, however, were no short-period increases of the progesterone concentration that was detected in the present study, probably because the twice-weekly determinations were too few to detect the transient increases in this hormone.

5. Conclusions

When goats are in Mediterranean latitudes, buck reproductive state is an important factor in determining the extent of response of does to the “male effect”. Subjecting bucks to 3 months of artificial long photoperiods in the winter prior to the spring when the “male effect” can be used to enhance goat reproduction, provides similar results as implanting bucks with melatonin around the time of the spring equinox. The photoperiod treatments of bucks represent a reliable alternative for farmers when there is prohibition of the use of hormonal treatments.

Conflicts of interest

None of the authors of this paper has any financial or personal relationship with any other person or organisation that might inappropriately influence or bias the content of the paper.

Funding

This study was funded by Grant AGL2016-75848-R from MINECO-AEI-FEDER (Spain).

Acknowledgements

The authors are grateful to CEVA Salud Animal, Barcelona, Spain, for providing the Melovine® implants.

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