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Efficacy of long day photoperiod treatment with respect to age of bucks for stimulation of the "male effect" on does at Mediterranean latitudes

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ABSTRACT

This study examines whether the photostimulation of sexual activity in young bucks improves the reproductive performance of the "male effect" in comparison to adult males. The experimental design was a 2 × 2 with to variables: age of bucks and photoperiodic treatment of bucks. Ninety-three anoestrous does were distributed into four groups depending on the kind of male used: young bucks (1.26 years old, $n = 6$) or old bucks (5.15 years old, $n = 6$). Half of each group of males were subjected to a photoperiodic treatment or a natural photoperiod. After the males were introduced, the sexual behaviour of the bucks was assessed for 10 days, and doe oestrous behaviour was recorded. Ovulation was confirmed from plasma progesterone concentration, and ovulation rate was assessed by transrectal ultrasonography. Fecundity, fertility, prolificacy and productivity were also determined. The females in contact with young bucks showed a higher percentage of ovulation (100% vs 81%, $P < 0.01$) and oestrous (82% vs 64%, $P < 0.05$) than females in contact with old bucks. The females in contact with photostimulated bucks showed higher percentages of oestrous (88% vs 60%, $P < 0.01$), fertility (78% vs 44%, $P < 0.01$) and productivity (1.08 ± 0.10 vs 0.60 ± 0.12 $P < 0.01$) than females in contact with control bucks. No interaction between both factors (age and photoperiod treatment) on any studied variable was observed. In conclusion, the response to the "male effect" was higher when using young bucks or photostimulated bucks. These photostimulated bucks produced 48 additional kids for every 100 females in the mating group compared to does exposed to untreated bucks.

1. Introduction

The seasonality of reproduction is a limiting factor on the productivity of the goat farm, which could be counterbalanced by the use of different environmental stimuli, such as socio-sexual interactions between males and females. Indeed, in seasonally anoestrous goats, sexual activity can be stimulated after joining with males; this phenomenon is called the "male effect" (Thimonier et al., 2000; Delgadillo et al., 2009). The percentage of does that ovulate when exposed to bucks can be dramatically modified by the sexual behaviour displayed by bucks. At Mediterranean latitudes, for example, the sexual behaviour of bucks is typically diminished from February to September, and consequently, the percentage of females that ovulate when joined with bucks is notably reduced during this period (Zarazaga et al., 2009; Gallego-Calvo et al., 2015). This lowered response by females can be overcome using males rendered sexually active by photoperiodic treatments. Indeed, the exposure of bucks to 2.5 or 3 months of artificially long days in autumn and winter, followed by natural photoperiod variations, stimulates their sexual activity during the sexual rest, and these sexually active bucks are more efficient than untreated bucks at inducing sexual activity in seasonal anoestrous does (Chemineau et al., 1988; Malpaux et al., 1989). Furthermore, these males are very efficient in activating the

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hypothalamic-pituitary-gonadal axis in anovulatory female goats (Delgadillo et al., 2002; Ponce et al., 2014).

Another factor that could modify the sexual response of females joined with males is the age of the bucks. In sheep, the use of adults, rather than young rams, is recommended to induce the reproductive activity of females, probably because adult rams display more sexual behaviours and produce more pheromones than young rams (Haynes and Haresign, 1987; Rosa and Bryant, 2002). In line with these hypotheses, Ungerfeld et al. (2008) reported that compared with yearling rams, adult rams induced ovulation, oestrus behaviour and pregnancy in a greater percentage of ewes. Other authors have demonstrated that sexually inexperienced rams from 8 or 21 months of age, without sexual contact with females, displayed lower sexual performances when exposed to females than males with continuous exposure to females (Katz et al., 1988; Price et al., 1991, 1994). Similarly, in goats, young bucks isolated from females since weaning showed lower rates of sexual behaviour when exposed to females (Lacuesta et al., 2018). Nonetheless, the sexually inexperienced bucks rendered sexually active by a photoperiodic treatment displayed intense sexual behaviour and induced more than 87% of seasonally anoestrous does to ovulated (Fernández et al., 2020).

Considering the results described in sheep and goats, we hypothesised that i) the young bucks could display a lower level of sexual behaviour than the adult males; and ii) that a photoperiodic treatment could override this difference, allowing the young bucks to induce the same percentage of females to ovulate as the old bucks. The objective of the current experiment was to compare, in opposition to an untreated control group, the efficacy of long-day photoperiod treatment on the response to the male effect of does, using young and adult bucks.

2. Materials and methods

2.1. Study conditions

All animals were handled in pens with an open territory and an enclosed zone. 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). All animals were fed Lucerne hay, barley straw (ad libitum) and commercial concentrate daily to maintain their body weight based on diets calculated using Institut National de la Recherche Agricole (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 supplement.

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

Four sets ($n = 3$ each) of sexually experienced Blanca Andaluza bucks were used to provoke the "male effect". The bucks of Blanca Andaluza showed an extended rest season determined by basal testosterone concentrations from December to July (Gallego-Calvo et al., 2014). Bucks were classified according to their age at the beginning of the study: young bucks (1.26 ± 0.03 years old, $n = 6$) and old bucks (5.15 ± 0.01 years old, $n = 6$). This breed shows complete sexual activity and good seminal quality when they are 1.4 years old (Gallego-Calvo et al., 2015). Within each age group, half of the males were kept under natural photoperiod variation conditions (untreated bucks, i.e. control), and half were submitted to photoperiodic treatment (photo group). On the 13th of November (sunrise at 8:06 and sunset at 18:20), two sets of young ($n = 3$) and old bucks ($n = 3$) housed in independent open outbuildings were exposed to artificially long days (16 h light: 8 h dark; lights on 6:00, lights off 22:00) for 88 days (i.e. the young photo and old photo groups). The photoperiod was managed by an electric clock that controlled white fluorescent strip lights giving around 200 lux at the height of the eyes of the bucks. Toward the end of the photoperiod treatment, i.e. on the 9th of February of the following year (sunrise at 8:25 and sunset at 19:01), these bucks were kept under natural photoperiod variations. The other young ($n = 3$) and old bucks ($n = 3$) were maintained under the natural photoperiod variation throughout the study (the young and old control groups). The bucks of each group were housed in isolated pens (Fig. 1).

2.3. Preparation of does

Ninety-three Blanca Andaluza multiparous anovulatory females were used in this study. Does were 3–5 years old at the beginning of the study. Parturitions occurred in these females between September and October of the previous year. The does were completely isolated from males from November and penned together until placement with the bucks, when the four groups were established.

2.4. The "male effect"

On the 2nd of April (D0) (sunrise at 8:13 and sunset at 20:51), 52 days after the end of the photoperiod treatment, bucks were equipped with marking harnesses and then placed with the experimental females for the following 32 days (until the 3rd of May; sunrise at 7:32 and sunset at 21:19). When bucks (three by group) were introduced with the females, they were divided into four groups of females according to age and photoperiodic treatment of the bucks: young photo ($n = 30$), young control ($n = 27$), old photo ($n = 20$), and old control ($n = 16$). Each group was housed in open barns, completely isolated from the animals in the other treatment groups (Fig. 1).

2.5. Variables recorded for the does

2.5.1. Detection of oestrous behaviour, ovulation and ovulation rate

To monitor the ovulatory cycles of does before placement with bucks (Day 0; the 2nd of April), blood samples were collected once per week for three consecutive weeks (16th, 23rd and 30th of March), and the plasma progesterone concentration was determined. Females with plasma progesterone concentrations ≤ 1.0 ng/mL in all samples before D0 were considered to be in anoestrous. Does with plasma progesterone concentrations ≥ 1.0 ng/mL in at least two consecutive samples were deemed to have ovulated and developed a corpus luteum of normal functional duration (Chemineau et al., 1992), and these does were discarded. No animals were removed following evaluation of the progesterone samples collected prior to the formation of the mating groups. 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 buck placement with does and the first detected oestrous behaviour was calculated for each doe.

After the introduction of bucks (D0), plasma progesterone concentration was determined twice per week (Mondays and Thursdays) to monitor the ovulatory response after male introduction. The date of onset of a normal ovulatory response was defined as that of the first sample with progesterone concentrations above baseline (≥1.0 ng/mL). Silent ovulation was deemed to have occurred when an increase in plasma progesterone above baseline was seen in at least one sample but was not preceded by oestrous behaviour. The percentages of females showing oestrus with or without ovulation, as well as those showing silent ovulation, were inferred from the plasma progesterone concentrations. The occurrence of ovulation and the ovulation rate was 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. Blood samples and hormone analysis

In all cases, blood samples from the jugular vein were collected in tubes with 10 μl of heparin. They were immediately centrifuged at

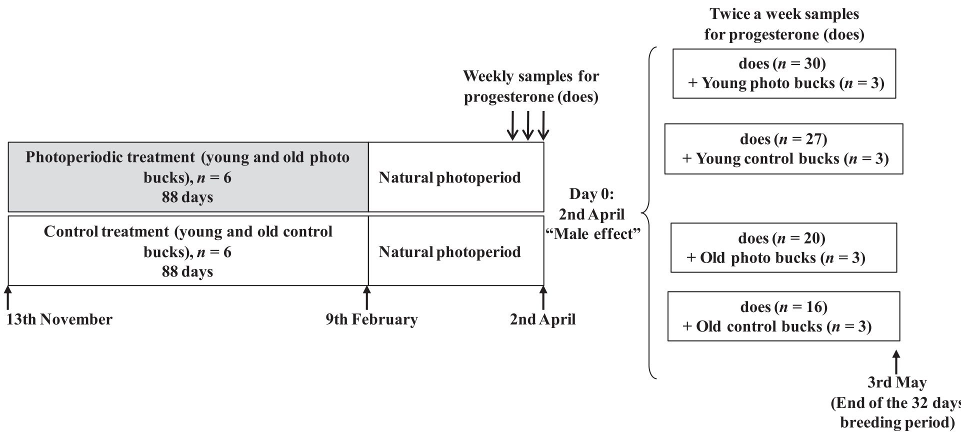

Fig. 1. Experimental design of the "male effect" of does using artificially treated bucks for long days for 3 months from November to February (photo bucks), or untreated males (control bucks). Half of each group were young bucks (1.26 ± 0.03 years old) and old bucks (5.15 ± 0.01 years old).

2300 × *g* for 30 min at 4°C, and the resultant plasma was stored at − 20 °C until 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 4.0%, 8.0%, and 6.1%, 8.0%, respectively.

2.5.3. Fecundity, fertility, prolificacy and productivity

Fecundity (percentage of pregnant does/does showing oestrus and ovulation) was determined using transrectal ultrasonography 45 days later from detection of oestrus (Schrick et al., 1993). Fertility (percentage of does kidding/does exposed to males), prolificacy (the number of kids born per female kidding) and productivity (the number of kids born per doe in each mating group) were also determined (Caravaca et al., 1999).

2.6. Buck plasma testosterone and sexual behaviour

Blood for the determination of plasma testosterone was obtained and managed as described previously (Section 2.5.2). Blood samples were taken weekly before day of introduction of bucks (D0) and twice a week after D0 at 9:00 until the end of the experiment (2nd of April – 3rd May). 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 4.7%, 4.1%, and 6.7%, 5.1%, respectively.

The sexual behaviour of the bucks in all groups was observed for 30 min each day at the same time (from 8:00–8:30) on Days 0–9 post-placement of bucks with does. Genital sniffs (buck sniffing the anogenital area of does), licks (buck licking the flanks of does), nudges (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 interruption to the animal behaviours.

2.7. Statistical analyses

The values for testosterone concentrations were examined using repeated measures ANOVA, and the model included fixed between-subjects experimental factors and a fixed within-subject factor for time (repeated measures), as well as the interactions between these factors. The linear model used for each parameter was as follows:

$$Y_{ijkl} = \mu + N_i + P_j + (N \times P)_{ij} + T_k + (T \times N)_{ki} + (T \times P)_{kj} + (T \times N \times P)_{kij} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the value for the dependent variable; μ is the overall mean; N_i is the fixed between-subjects effect of the age of the bucks (i = young or old bucks); P_j is the fixed between-subjects effect of photoperiod treatment of the bucks (j = photo or control); T_k is the within-subject fixed effect of time; $N \times P$, $T \times N$, $T \times P$, $T \times N \times P$ are the interactions among these factors; and ε_{ijkl} is the residual error. The Tukey test was used to detect weekly differences between groups.

Productivity and the number of days between male introduction and ovulation, or ovulation with oestrous behaviour, were compared using ANOVA with age and the male treatment as fixed effects. The linear model used for each parameter was as follows:

$$Y_{ijk} = \mu + P_i + M_j + (P \text{ x } M)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the value for the dependent variable; μ is the overall mean; is the fixed between-subjects effect of the age of the bucks (i = young or old bucks); P_j is the fixed between-subjects effect of photoperiod treatment of the bucks (j = photo or control); $(N \text{ x } P)_{ij}$ is the interaction among these factors, and ε_{ijk} is the residual error.

The variables expressed as percentages—does with ovulations, those expressing oestrous behaviour and having ovulation, fecundity and fertility—were analysed using the multinominal logistic regression and the Fisher exact probability test for two-group comparisons as required. Ovulation rates and prolificacy were compared using the Mann–Whitney U test.

The percentages of genital sniffs, licks, nudges, sneezing sounds, mounting attempts, and mounting with intromission were calculated for each group and 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.

Data are expressed as the mean ± SEM (standard error of the mean), and differences were considered significant at $P \leq 0.05$. All calculations were performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA).

2.8. Ethical note

All methods were performed via prepared human resources in exact understanding with Spanish rules for the insurance of investigational animals (RD 53/2013), and in concurrence with European Union Directive 86/609. The techniques of the current trial were assessed by the certified association of the ethical committee for animal experimentation (CEEA-OH) from the University of Granada and approved with the reference number 297-CEEA-OH-2018 and authorised by the Andalusia Regional Government with the number 22/05/2019/094. 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).

3. Results

3.1. Doe reproductive response

The percentage of females showing ovulation via the elevated progesterone concentration or showing oestrus with ovulation and productivity was higher in the females in contact with the young bucks (at least P < 0.05; Table 1). The age of the bucks did not modify any other reproductive parameter studied. The percentage of females showing oestrus with ovulation, fertility and productivity was higher in the groups of females exposed to photo bucks compared to the females in contact with control bucks (P < 0.01; Table 1). The interaction buck age × photoperiod treatment did not affect any of the reproductive variables studied (P > 0.05; Table 2).

Table 1
Reproductive response of does submitted to the "male effect" using young bucks (1.26 ± 0.03 years old, *n* = 6) and old bucks (5.15 ± 0.01 years old, *n* = 6). Half of each age group were photoperiod-treated males (photo, *n* = 6), and half were males subjected to the natural photoperiod (control, *n* = 6). Effect of two main factors.

Variable	Old (*n* = 36)	Young (*n* = 57)	Photo (*n* = 50)	Control (*n* = 43)
Females ovulating (%)	81B	100A	94	91
Interval introduction of male- normal ovulation (days)	11.3 ± 0.8	10.8 ± 0.4	11.0 ± 0.5	10.9 ± 0.5
Females in oestrus and ovulating (%)	64b	82a	88A	60B
Interval between introduction of male and oestrus (days)	8.0 ± 0.7	6.8 ± 0.4	7.6 ± 0.6	6.6 ± 0.5
Ovulation rate (corpora lutea)	1.42 ± 0.10	1.44 ± 0.08	1.48 ± 0.08	1.38 ± 0.10
Fecundity (%)	96	91	95	88
Fertility (%)	56	67	78 A	44B
Prolificacy (kids born by female kidding)	1.40 ± 0.11	1.33 ± 0.08	1.38 ± 0.08	1.29 ± 0.12
Productivity (kids by number of females in the group)	0.78 ± 0.13b	0.91 ± 0.10a	1.08 ± 0.10A	0.60 ± 0.12B

a, b: Different letters in the same row within each variable reflect significant differences at P < 0.05.
A, B: Different letters in the same row within each variable reflect significant differences at P < 0.01.
Females ovulating (percentage of does at least with one progesterone concentrations above baseline ≥1.0 ng/mL).
Interval introduction of male- normal ovulation (interval between buck placement with does and the first progesterone concentrations above baseline ≥1.0 ng/mL).
Females in oestrous and ovulating (percentage of does showing oestrous with at least one progesterone concentration above the baseline ≥1.0 ng/mL).
The interval between the introduction of males and oestrus (interval between the time of buck placement with does and the first detected oestrous).
Ovulation rate (number of corpora lutea observed in each doe by transrectal ultrasonography conducted 6–8 days after the detection of oestrus).
Fecundity (percentage of pregnant does/does showing oestrus and ovulating) was determined using transrectal ultrasonography on day 45 after oestrus was detected.
Fertility (percentage of does kidding/does exposed to males).
Prolificacy (the number of kids born per female kidding).
Productivity (the number of kids born per doe in each mating group).

Table 2
Reproductive response of does submitted to the "male effect" using young bucks (1.26 ± 0.03 years old, *n* = 6) and old bucks (5.15 ± 0.01 years old, *n* = 6). Half of each age group were photoperiod-treated males (photo, *n* = 6), and half were males subjected to the natural photoperiod (control, *n* = 6). Interaction between the two main factors.

	Young Control (*n* = 27)	Old Control (*n* = 16)	Young Photo (*n* = 30)	Old Photo (*n* = 20)	Interaction
Females ovulating (%)	100	75	100	85	NS
Interval introduction of male-normal ovulation (days)	10.3 ± 0.6	12.1 ± 1.1	11.2 ± 0.5	10.8 ± 1.1	NS
Females in oestrus and ovulating (%)	70	44	93	80	NS
Interval between introduction of male and oestrus (days)	5.9 ± 0.6	8.4 ± 0.6	7.5 ± 0.6	7.8 ± 1.1	NS
Ovulation rate (corpora lutea)	1.40 ± 0.12	1.33 ± 0.17	1.48 ± 0.10	1.47 ± 0.13	NS
Fecundity (%)	89	86	93	100	NS
Fertility (%)	52	31	80	75	NS
Prolificacy (kids born by female kidding)	1.25 ± 0.14	1.40 ± 0.25	1.38 ± 0.10	1.40 ± 0.13	NS
Productivity (kids by number of females in the group)	0.70 ± 0.15	0.44 ± 0.18	1.10 ± 0.13	1.05 ± 0.17	NS

Females ovulating (percentage of does at least with one progesterone concentrations above baseline ≥1.0 ng/mL).
Interval introduction of male- normal ovulation (interval between buck placement with does and the first progesterone concentrations above baseline ≥1.0 ng/mL).
Females in oestrous and ovulating (percentage of does showing oestrous with at least one progesterone concentration above the baseline ≥1.0 ng/mL).
The interval between the introduction of males and oestrus (interval between the time of buck placement with does and the first detected oestrous).
Ovulation rate (number of corpora lutea observed in each doe by transrectal ultrasonography conducted 6–8 days after the detection of oestrus).
Fecundity (percentage of pregnant does/does showing oestrus and ovulating) was determined using transrectal ultrasonography on day 45 after oestrus was detected.
Fertility (percentage of does kidding/does exposed to males).
Prolificacy (the number of kids born per female kidding).
Productivity (the number of kids born per doe in each mating group).

3.2. Testosterone concentrations and sexual interactions with bucks

Time had a marked effect on the testosterone concentration of the plasma (P < 0.01), as did the interaction of time × buck photoperiodic treatment (P < 0.01). The testosterone concentrations of the photoperiod-treated bucks (photo group) diminished rapidly after the onset of treatment. This group had greater testosterone concentrations than the bucks of the control group from the 16th of March until the 16th of April (Fig. 2), except for two samples, which occurred at the same time as the bucks were placed in contact with does for induction of the "male effect". None of the main factors, the age of the bucks or the photoperiod treatment or any interaction between them, affected the testosterone concentrations (P > 0.05).

The photo group bucks performed more genital sniffing, made more sneezing sounds and completed more mounts with intromission than the

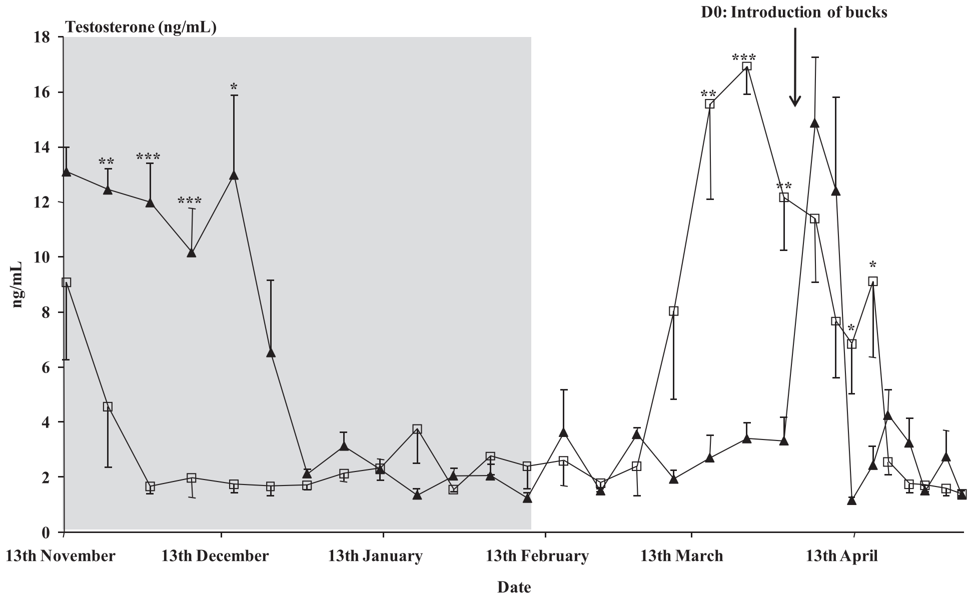

Fig. 2. Plasma testosterone concentration (ng/mL) of bucks [mean values of young (average 1.26 years- males) and old (average 5.15 years)] submitted to a photoperiodic treatment, i.e. long days for 3 months between November and February (photo, line with □) or not (control, line with ▲). The arrow indicates the moment when the "male effect" was performed. The shadow area indicates the time of the photoperiod treatment. * P < 0.05; ** P < 0.01; *** P < 0.001.

control bucks (at least P < 0.05), but the control bucks had more mounting attempts (P < 0.01; Fig. 3). The young bucks performed more genital sniffing, licking, and made more mounting attempts and mounts with intromission (P < 0.01), while the old bucks made more sneezing sounds (P < 0.01; Fig. 4).

4. Discussion

Our results show that the age and the photoperiodic treatment of the bucks, which can modify the sexual activity of bucks, are significant and independent factors when looking for the greatest response to the "male effect" in spring. Better reproductive responses and performances were seen in the set of females submitted to the "male effect" when young bucks were used and in those exposed to bucks treated with photoperiod, but those factors were independent because no interaction between them was observed.

The first remarkable result was the very high percentage of females (greater than 90%) showing elevated progesterone concentrations after the introduction of males, independent of the age or the photoperiodic treatment received by the males. However, there was a higher response in the females in contact with young males (100% females ovulating). Flores et al. (2000) and Delgadillo et al. (2002) observed hardly any elevation of progesterone after teasing using bucks who were subjected to the natural photoperiod. Different and non-exclusive explanations could be suggested. Firstly, we used a very high male:female ratio, between 1:5 and 1:10. Nevertheless, in a recent experiment using photostimulated males, we observed a similar ovulation response at male: female ratios between 1:5 and 1:20 (Zarazaga et al., 2018). The high male:female ratio could not explain this difference with the results of Flores et al. (2000) and Delgadillo et al. (2002) (in general, they used a 1:10 ratio). Perhaps the importance of the male:female ratio is greater when the males are not photostimulated. Secondly, the introduction of the males could induce reactivation of the hypothalamus-pituitary-gonadal axis that induced an increase in progesterone concentrations. However, this reactivation did not induce an adequate oestrous response because the oestrous response diminished by around 30% in the female groups in contact with the control males. A reduction in the percentage of females showing oestrous compared to

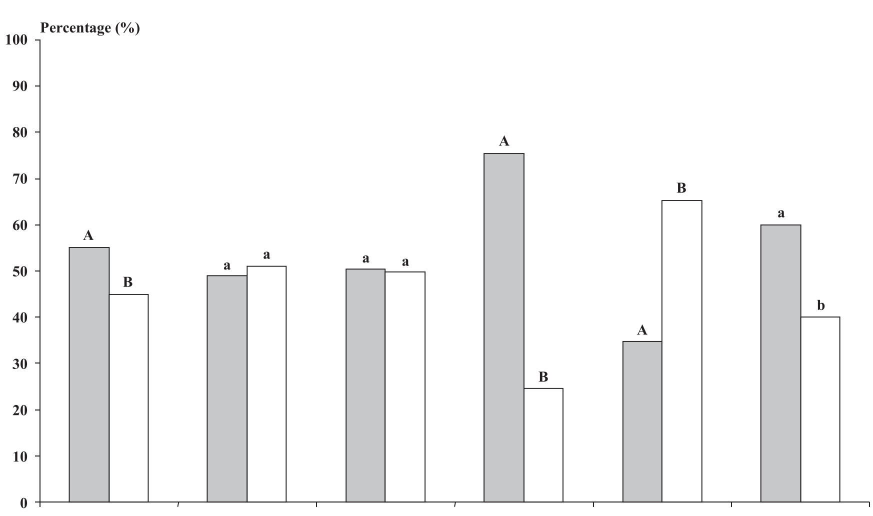

Fig. 3. Types of sexual advances (%) performed by the males when females were subjected to the "male effect" using photoperiod-treated males (■, $n = 6$) or males exposed to the natural photoperiod (□, $n = 6$). Half of each group were young bucks (1.26 ± 0.03 years old) and old bucks (5.15 ± 0.01 years old). Different letters in the same sexual advance indicate a significant difference: a, b: (P < 0.05); A, B: (P < 0.01). Sniffs (when the buck sniffed the anogenital area of the doe). Licks (when the buck licked the flanks of the doe). Nudges (when the buck kicked the doe). Sneezing sounds (bucks emitting a sneezing sound). Mounting attempts (when the buck attempted to mount the doe without intromission). Mounting with intromission (when the bucks mounted the doe with intromission).

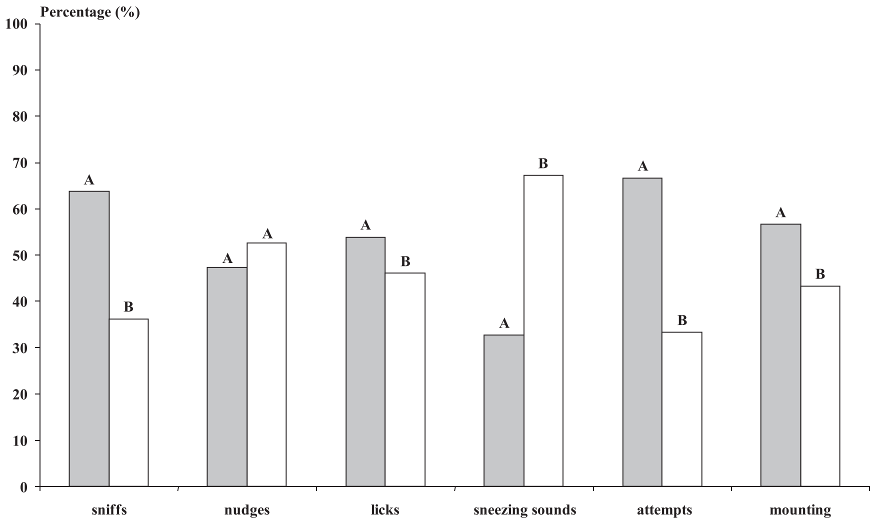

Fig. 4. Types of sexual advances (%) performed by males when females were subjected to the "male effect" using young bucks (1.26 ± 0.03 years old, ■, $n = 6$) and old bucks (5.15 ± 0.01 years old, □, $n = 6$). Half of each group were photoperiod-treated males or males exposed to the natural photoperiod. Different letters in the same sexual advance differ significantly (A, B: $P < 0.01$). Sniffs (when the buck sniffed the anogenital area of the doe). Licks (when the buck licked the flanks of the doe). Nudges (when the buck kicked the doe). Sneezing sounds (bucks emitting a sneezing sound). Mounting attempts (when the buck attempted to mount the doe without intromission). Mounting with intromission (when the bucks mounted the doe with intromission).

the percentage of females ovulating was always observed. Thirdly, the introduction of the males reactivated the hypothalamus-pituitary-gonadal axis, but the lower number of interactions with the females in the groups could have induced a lower oestrous response in the females. Moreover, in this reproductive parameter, a clear effect of the age of the buck was observed, with a lower percentage of females in contact with older bucks showing ovulation. The most plausible reason for this lower reproductive response in those groups could be the lower male-female interactions observed in the old groups, except for the sneezing sounds.

The percentage of females showing oestrous and productivity were higher in the groups with young bucks. These results contrast with results obtained by Ungerfeld et al. (2008) in ewes. Those investigators saw that mature rams induced a higher percentage of ovulation and oestrous response, bringing about a higher number of pregnancies and conception rates in anoestrous ewes than when yearling rams were used. These authors propose that this more important stimulation could be, to some degree, due to the signs, probably odours, given in the wool of mature rams. We did not evaluate the odour of the males, and as a consequence, we cannot determine if this parameter was different between groups of males and, in the present experiment, favourable for the young bucks. Ungerfeld et al. (2008) conjectured that the lesser level of pregnancies achieved when yearling rams were used could be clarified by differences in mounting practices and ejaculation frequency. This explanation does not agree with our results. In our experiment, the young males experienced more interactions with the females than the old males. Except for the number of sneezing sounds, the other interaction parameters with the females were more frequent in the groups with young bucks than with old bucks. Despite the difference between groups of male ages observed in the percentage of females showing oestrous, age groups had no observable effect on fertility, suggesting that the number of fertile ejaculations was not less in the young bucks. Moreover, productivity was higher in this group.

The higher productivity of the does in contact with young bucks can not be explained by the higher fecundity or fertility of these does because no differences between groups were observed. These results could indicate that the age of bucks in the present experiment was not a critical factor in the fertility and fecundity of the does that showed oestrous. To our knowledge, no effect of the age of buck has been observed on fertility, but a clear effect of the age of does has been demonstrated (David et al., 2008; Arrebola et al., 2013). However, in other species such as birds, age has an adverse effect on reproductive success, diminishing fertility (Bramwell et al., 1996; Tabatabaei et al., 2010).

The females grouped with photostimulated males showed higher percentages of oestrus, fertility and productivity (+48 kids per 100 goats in the mating group). This result is similar to other results published in the literature (Flores et al., 2000; Delgadillo et al., 2002). This corroborates that the male reproductive condition is essential to provoke a high reproductive response to the "male effect" in female goats. The better results of the female groups in contact with photostimulated bucks could be due to the higher exploratory activity of these bucks because they showed a higher number of anogenital sniffs and a much higher number of sneezing sounds. The higher number of interactions of these bucks could be explained as due to an increase in testosterone concentration from the artificially long days during what would usually be the period of sexual inactivity for goats at Mediterranean latitudes. Photoperiod-treated bucks are therefore able to induce higher reproductive performances in does, increasing the profitability in comparison to the use of males with natural sexual activity for that time of year.

This higher reproductive performance fertility and productivity could perhaps be explained by the bucks submitted to the artificial photoperiod having higher sperm quality than the bucks of the control group. However, our group worked with males of the same breed (Gallego-Calvo et al., 2015) observed that a rapid succession of two months of long days (LD) and short days (SD) induced higher values for sperm concentration and total sperm number per ejaculate during the LD treatment. Annual variation in these parameters has been described in the literature for different goat breeds (Karagiannidis et al., 2000; Pérez and Mateos, 1996; Roca et al., 1992).

5. Conclusions

The present results show that at Mediterranean latitudes, the age of the bucks used to induce the "male effect" and the reproductive condition of the bucks were independent because no interaction between them was observed. The percentages of female goats ovulating, showing oestrus and productivity after a "male effect" induced using young bucks is stronger than those using adult bucks. The does mated with photostimulated bucks produced 0.48 kids more per doe in the mating group than the does exposed to untreated bucks. These facts lead to the conclusion that to obtain a high reproductive efficiency on goat farms, it is desirable to use experienced young bucks or photostimulated bucks of any age.

CRediT authorship contribution statement

L.A. Zarazaga: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Supervision, Project administration, Funding acquisition, **M.C. Gatica:** Formal analysis, Data curation, Writing – review & editing, **M. Delgado-Pertíñez:** Formal analysis, Data curation, Writing – review & editing, **Hernandez H. Hernández:** Formal analysis, Data curation, Writing – review & editing, **J.L. Guzmán:** Methodology, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition, **J.A. Delgadillo**: Conceptualization, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author, LAZ, upon reasonable request.

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Disclosure statement

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