When using photostimulated bucks to induce the male effect in female goats living at Mediterranean latitudes, a male:female ratio of 1:20 is optimum

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Abstract
This study examines the reproductive response and reproductive performance of does subjected to the male effect at different male:female ratios when photostimulated males are used. One hundred and thirty does were distributed homogeneously into six groups with male:female ratios of either 1:30 (two repetitions of 30 females with one male each), 1:20 (20 females with one male), 1:15 (15 females with one male), 1:10 (20 females with two males), or 1:5 (15 females with three males). After the introduction of the males (all made sexually active by keeping them for three months under long days), oestrous activity was recorded daily by direct visual observation of the marks left by marking harnesses worn by the males. Ovulation was confirmed via the plasma progesterone concentration. Fecundity, fertility, prolificacy and productivity values were also determined. The 1:30 group returned the lowest percentage of does that ovulated and that showed oestrous activity; it also returned the lowest fecundity and fertility values. The highest values for all these variables were recorded for the 1:5–1:20 groups (with no significant difference between them). These results show that, under Mediterranean latitudes, the reproductive response, fecundity and fertility are diminished when the photostimulated male:female ratio is very low (1:30). The optimum ratio would appear to be around 1:20.

Introduction
Reproductive seasonality is commonly seen in goats living at temperate and subtropical latitudes. Does are normally receptive from early autumn to late winter (Chemineau et al. 1992; Gómez-Brunet et al. 2003; Zarazaga et al. 2005; Gallego-Calvo et al. 2014) while bucks show intense sexual behaviour from late summer to late winter (Delgadillo et al. 1999; Zarazaga, Guzmán, Domínguez, Pérez and Prieto 2009; Gallego-Calvo et al. 2015). On farms, however, year-round production is required; thus, females need to be brought into oestrus during the anoestrus period by males displaying sexual behaviour (made to do so if necessary).

The problem of seasonality in does is commonly overcome via the manipulation of sociosexual relationships known to have an impact on the onset of reproductive activity (Hutchinson 1991; Martin et al. 2004; Rodríguez-Martínez et al. 2013). For example, introducing a male to a group of females induces and synchronizes their sexual activity: a phenomenon known as the ‘male effect’ (Delgadillo et al. 2009).

At Mediterranean latitudes, anoestrus lasts from January–March to August–September (Zarazaga et al. 2005; Gallego-Calvo et al. 2014), whereas bucks experience a reduction in their testosterone levels and sexual behaviour between January–February and June–July (Zarazaga et al. 2010; Gallego-Calvo et al. 2015). Using bucks for the male effect during this period induces ovulation in very few does (Flores et al. 2000; Chasles et al. 2016). This problem, however, can be circumvented by using bucks made sexually active via the appropriate photoperiod treatment. Indeed, all does exposed to photostimulated males may ovulate, compared to under 10% when non-photostimulated, sexually inactive males are used (Delgadillo et al. 2002; Chasles et al. 2016). At Mediterranean latitudes, three months of long days between the second fortnight of November and the second fortnight of February, followed by natural photoperiod conditions, increases plasma testosterone concentrations and improves male sexual behaviour in March–April (Zarazaga et al. 2010).

The male:female ratio has been reported to influence the sexual response of does to the male effect (Signoret et al. 1982; Chemineau 1987). When this ratio was reduced from 1:10 to 1:20 and then 1:100, the response rate dropped from 87% to 80% and finally 25% (Signoret et al. 1982; Cushwa et al. 1992; Walkden-Brown et al. 1993a). Carrillo et al. (2007), however, who used photostimulated bucks, observed no negative effect of reducing the male:female ratio from 1:10 to 1:39 in terms of the ability to induce oestrous activity in anoovulatory female goats – although a delay in female response was seen at the lowest male:female ratio.

The present working hypothesis was that, under Mediterranean conditions, the response to the male effect depends on the number of females per male. The aim of the present work was to determine whether the male:female ratio affects the...
response of does to the male effect when the males used are sexually active (having undergone three months of long-day photoperiod treatment.

Material and methods

Study conditions

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 study was conducted at the University of Huelva experimental farm (latitude 37°15′N), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

Animals and management

The females used in this work were adult (3–4 years old), non-pregnant Blanca Andaluza/Payoya does (n = 130). Over the entire experimental period, the does were maintained indoors under natural photoperiodic conditions and fed daily with lucerne hay, barley straw and commercial concentrate according to INRA standards for maintaining adult weight and for providing adequate nutrition (Morand-Fehr and Sauvant 1988). All animals had free access to water and mineral blocks containing trace elements and vitamins.

Before introduction to the males (Day 0; April 4th), blood samples were collected from all females once per week over four consecutive weeks to determine their plasma progesterone concentrations (see Section 2.5 for technical details). Any female with a plasma progesterone concentration of > 1.0 ng/mL in at least two consecutive samples was deemed cyclic and excluded from the study.

Preparation of females and males

Females

Figure 1 shows the experimental protocol. Even before the 15th October starting date of the experiment, the females had been maintained completely isolated from all males. On 4th April, the females were allocated to one of the following male:female ratio treatment groups: 1:30 (two repetitions [given this very low ratio] of 30 females in contact with one photostimulated male each), 1:20 (20 females in contact with one photostimulated male), 1:15 (15 females in contact with one photostimulated male), 1:10 (20 females in contact with two photostimulated males), or 1:5 (15 females in contact with three photostimulated males). The number of Blanca Andaluza and Payoya does was the same within each group. These six groups remained in shaded open pens under natural day length conditions for 36 days.

Male

Fifteen males were exposed to 3 months of long days (16 h of light per day) from November 19th until 15th February, followed by 1.5 months exposure to natural photoperiod conditions. Long days were ensured by providing natural light plus artificial light from 06.00 to 08.00 h and from 18.00 to 22.00 h. The intensity of the artificial light was at least 300 lux at the animals’ eye level.

Blood for the determination of plasma testosterone was obtained by jugular venipuncture and using vacuum tubes containing heparin. Blood was sampled weekly at 09:00 h from the onset of the photoperiod treatment until the end of the experiment. Plasma was obtained by centrifugation at 3500 g for 30 min, and stored at −20°C until use. Testosterone concentrations were determined using a commercial enzyme-linked immunoassay (ELISA) kit (Demeditec Diagnostics, Kiel-Wellsee, Germany). The intra- and inter-assay coefficients of variation were 4.3% and 5.0%, respectively.

The male effect

One week before the male introductions, checks were made that the 15 bucks showed sexual behaviour (nudging, ano-genital sniffing, mounting attempts, and flehmen reaction) by exposing them for 5-min periods to oestrous females (not those used in the experiment). Nine males that displayed similar sexual behaviour were then randomly allocated to the six treatment groups on April 4th (Day 0) (Figure 1) and left in contact with the does for 36 days (May 9th).

Female response to the male effect

Detection of oestrous behaviour

Oestrous activity was recorded by daily visual observation of the marks left by the marking harnesses worn by the bucks (Walkden-Brown et al. 1993b).

Ovulation was detected by the presence of corpora lutea observed by transrectal ultrasonography conducted 6–8 days after the detection of oestrous activity (Simoes et al. 2005). The presence of corpora lutea was confirmed by monitoring plasma progesterone. Blood samples were taken twice weekly from the time of introduction to the males until the end of the study. All samples were collected by jugular venipuncture and using tubes containing 10 µL of heparin. Plasma was obtained by centrifugation at 3500 g for 30 min and stored at −20°C until hormone measurements were made. Plasma progesterone was determined in duplicate samples using a commercial ELISA kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer’s instructions (Madgwick et al. 2005). The mean intra-assay and inter-assay coefficients of variation were 6.1% and 9.1%, respectively. The sensitivity of the assay was 0.2 ng/mL. Females with progesterone concentrations of ≥ 0.5 ng/mL were considered to have ovulated (Che-mineau et al. 1984; Zarazaga, Guzmán, Domínguez, Pérez, Prieto and Sánchez 2009).

Fecundity, fertility, prolificacy and productivity

Fecundity (pregnant does/does in the group) was determined via transrectal ultrasonography on day 45 after the onset of oestrus in each female (Schrick et al. 1993). Fertility (percentage of goats kidding/does in the group), prolificacy (number of kids born per female kidding) and productivity (number of kids born per female in each mating group) values were calculated as shown.
Statistical analysis

Results are reported as means ± standard error. The percentage of females showing ovulation, oestrous activity and ovulation, and the fecundity and fertility values, were compared using the Chi-squared test or Fisher’s exact probability test as required. Prolificacy was compared using the Mann–Whitney U test. Productivity and the time elapsed between male introduction and ovulation/oestrous activity were compared using one-way ANOVA with the five experimental groups as fixed effects. Testosterone concentrations were examined by ANOVA with time as a repeated measure and the five experimental groups as main factors. When differences between groups were observed, a Tukey test was performed. Significance was set at $P < .05$. All analyses were performed using the SPSS package (IBM 2012).

Results

No differences were seen between the results for the repetitions of the 1:30 ratio; they were therefore considered as one in all analyses.

Table 1 shows that the response to male introduction was modified by the male:female ratio. The 1:30 group returned the lowest percentage of females showing ovulation and oestrous activity, while the highest percentages were shown by the 1:5–1:20 groups (with no significant differences between these latter groups). The fecundity and fertility values were also at their lowest in the 1:30 group and highest in the 1:5–1:20 groups (with no significant differences between these latter groups). Prolificacy was greatest in the 1:15 group and lowest in the 1:10 and 1:20 groups. No differences were seen between the groups in terms of productivity (0.73 ± 0.08 kids born per female). Finally, no differences were seen in the time elapsed between male introduction and ovulation or oestrous activity.

Figure 2 shows a significant ($P < .001$) effect of time on the testosterone concentrations of the used males. Testosterone concentrations decreased when the photoperiod treatment started, and increased just before the bucks were used to induce the male effect. Neither the group of does, nor the interaction group × time had any effect on buck testosterone concentration during the experimental period.

Discussion

The present results show that, at Mediterranean latitudes, bucks made sexually active by three months of long days can efficiently induce ovarian and oestrous activity in does when the male:female ratio is up to 1:20. The 1:5–1:20 ratios induced a significantly stronger reproductive response and higher fecundity and fertility values than did the 1:30 ratio. These results could be useful for Mediterranean goat production systems, including for organic goat production in which no hormones are allowed.

The ovulation response in all groups was very strong, with around 80% showing ovulation, and most ovulating animals showing oestrous activity as well. Several studies report that...
photostimulated bucks stimulate the sexual activity of does much better than bucks in sexual rest (Flores et al. 2000; Delgadillo et al. 2002; Bedos et al. 2014). Indeed, it has recently been shown in goats (Delgadillo et al. 2015) and sheep (Abecia et al. 2015) that the use of sexually active males stimulates does so effectively that anoestrus can almost be considered a solved problem.

Nevertheless, ovarian and oestrous activity was significantly lower at the lowest male:female ratio (1:30) than at all other ratios. The results obtained using the highest male:female ratios (1:5–1:20) are similar to those obtained by Angel-Garcia et al. (2015) and Carrillo et al. (2007) who used the same range. However, the results obtained for the present lowest ratio (1:30) contrast with those for the lowest ratio used in the Carrillo et al. (2007) study (1:39). Indeed, the authors of the latter work indicate that reducing the male:female ratio does not reduce the ability of sexually active bucks to induce oestrous activity in anovulatory does. This discrepancy could be due to differences in the breed used (Blanca Andaluza and Payoya in the present work, and Creole in the work by Carrillo et al.), as has been described in sheep (Nugent et al. 1988). Certainly, the males used in both experiments showed the same level of sexual activity. Moreover, the time of the year when both experiments was performed was similar.

The fecundity and fertility values obtained were lowest when the lowest (1:30) male:female ratio was used. It may be that when the number of females per male is increased, the pheromone signals perceived by the females are diluted, inducing a weaker ovarian and oestrous response and consequently fewer pregnancies (Carrillo et al. 2007). Alternatively, when there is larger number of females, the attention of the males might be concentrated on the most dominant females (Rodríguez-Iglesias et al. 1991; Álvarez et al. 2003), leaving some females unattended to. A larger number of females might also cause a reduction in the bucks’ semen quality, a consequence of short recovery periods between matings. However, the latter is unlikely to be responsible for the present results since, when the number of females deemed pregnant or that kidded is compared to the number that showed signs of oestrous activity, no differences are seen between the groups (61% of all females in all groups that showed oestrous activity became pregnant or kidded). This suggests that neither semen production nor its quality was a limiting factor in achieving pregnancies. Thus, males exposed to long days not only show improved sexual behaviour, but their production of sperm, and its quality, is sufficient to induce pregnancy during spring.

Finally, productivity was not affected by the male:female ratio. This may seem surprising since all the other reproductive variables examined were affected at the 1:30 ratio. However, this might be explained mathematically in that the prolificacy of all groups was very high, but lowest for the 1:10 and 1:20 groups.

Conclusions

The present results show that bucks made sexually active by exposure to long days, induce a better reproductive response and performance in females if the male:female ratio is 1:5–1:20. However, if this ratio falls to 1:30, the number of females ovulating or showing oestrous activity, and the values achieved for fecundity and fertility, clearly fall. It is, therefore, recommended that, at Mediterranean latitudes, one photoperiod-stimulated buck be provided per 20 does for optimum female reproductive performance to be achieved with the minimum number of males.
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Disclosure statement

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