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The isolation of females from males to promote a later male effect is unnecessary if the bucks used are sexually active

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ABSTRACT

It has been suggested that female goats in permanent contact with males become refractory to their presence, and need to be previously separated from them for 40–45 days if the presence of bucks is to induce reproductive activity, ovulation and oestrous during seasonal anoestrous. The present study examines the reproductive response (ovulation and oestrus) and reproductive performance of does isolated from bucks for different periods before their reintroduction to male company. A total of 103 Payoya and Blanca Andaluza does were distributed into six treatment groups that required their isolation from males for different periods: 0 days (N = 29), 5 days (N = 15), 10 days (N = 14), 20 days (N = 16), 30 days (N = 14) and 39 days (N = 15). After this period they were introduced to sexually active bucks (ensured to be in this condition by keeping them under long days light treatment for three months), and oestrous activity was recorded daily by direct visual observation of the marks left by the marking harnesses worn by these males. Ovulation was confirmed via the plasma progesterone concentration (measured in blood samples taken twice per week). The ovulation rate was assessed by transrectal ultrasonography. Fecundity, fertility, prolificacy and productivity were also determined. The sexual behaviour of the males towards the females was also monitored on Days 0, 1, 2, 3, 4, 8 and 9 after their meeting with the latter. The length of the female isolation period had no effect on the percentage of does that responded to contact with the males, nor did it affect the oestrous response, fecundity, fertility or productivity. The males, however, undertook more ano-genital sniffing and nudging with the 5 day group females compared to those of the other groups ($P < 0.05$). However, the sexual behaviour of the males changed as the days passed, with ano-genital sniffing becoming less common, and nudging, licking, sneezing and mounts with intromission more frequent on Days 8 and 9 than on Day 0, 1 and 2 after the sexes were reunited ($P < 0.05$). These results show that the isolation of females is not necessary for an efficient male effect if the bucks used are sexually active. In addition, the sexual behaviour of the bucks changes as the time in contact with the does increases, but in general is not affected by the duration of female isolation.

1. Introduction

The reproductive seasonality of goats living in subtropical and temperate latitudes is an important limitation to productivity. To counter this, reproduction needs to be induced during natural seasonal anoestrus [1–5]. The induction of the male effect via the re-introduction to males of does isolated from bucks has been shown an effective means of inducing female reproductive activity during this time of normal sexual rest [6–11]. In fact, the sudden exposure of anovulatory does to bucks results in a rapid increase in their luteinising hormone (LH) pulse frequency, followed by a pre-ovulatory LH surge and ovulation [7,8]. It has been suggested, however, that the duration of isolation from males, and the intensity of male sexual behaviour upon new contact, may influence the response of does [8].

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Underwood et al. (1944) [12] were the first to propose that that anoestrous ewes in permanent contact with rams likely become refractory to male stimuli, and that they respond to rams only if conditioned by a period of total isolation from all male contact. Thereafter it was shown that a period of isolation from rams of 17–21 days was enough to induce ovulation upon the resumption of contact [13,14]. However, just 24 h of isolation was sufficient to increase the LH pulsatility of ewes [15]. Shelton (1960) [16] then described the effect of introducing male goats to a group of does isolated from bucks at the end of seasonal anoestrus. Later, Chemineau (1987) [7] suggested that an isolation period of at least 3 weeks was necessary to induce ovulation in does re-exposed to bucks. Together, these findings suggest that, in both goats and sheep, females must be isolated from males if ovulation is to be induced via the male effect [8]. However, it has been suggested that such female isolation is not necessary when sexually active males are used [10,17]. In addition, the ovulatory response of does to the male effect does not depend on male novelty [18]; in the latter work, a high proportion of does ovulated when exposed to either novel or familiar males - if both were sexually active.

The degree of sexual activity displayed by males in spring - when the male effect is employed - might influence the response of females to their presence. Bucks from Mediterranean and subtropical latitudes show a strong reduction in their plasma testosterone concentrations in spring, and consequently display only weak sexual behaviour from December to July (the months corresponding to the natural sexual rest period) [19–21]. Certainly, some authors report bucks employed during the sexual rest period to induce only a low percentage of does to ovulate [22,23]. This limitation of the male effect can be circumvented by the use of bucks made sexually active by adequate photoperiod treatment. Experiments have shown that under such circumstances all previously isolated does exposed to photostimulated males ovulate, whereas <10% may do so when exposed to non-treated, sexually inactive males [23,24]. Interestingly, in Mediterranean latitudes, three months of long days between the second fortnight of November until the second fortnight of February, followed by natural photoperiod conditions, also increases buck plasma testosterone concentrations and intensifies their sexual behaviour in March-April [20].

Given the capacity of adequately photostimulated bucks to induce ovulatory activity in does in seasonal anoestrus, it was hypothesized that prior female isolation may not be necessary for reproductive activities in such does to be stimulated. Further, the characteristics of this reproductive activity might not differ regardless of the duration of female isolation from males. These ideas were tested by monitoring the ovulatory/oestrous activities and reproductive performances of does exposed to males after different periods of separation.

2. Material and methods

The study was conducted at the University of Huelva experimental farm (37° 20′N, 6° 54′ W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63).

2.1. Animals and management

The females used in this work (Payoya and Blanca Andaluza goats) were 3–4 year-old (adult) non-pregnant does ($n = 103$). At the latitude where the work was performed, female anoestrus lasts from January–March to August-September [3,5], while male sexual rest lasts from January–February to June-July [21,25].

Over the experimental period, the does were maintained indoors and fed daily with lucerne hay, barley straw and commercial concentrate, according to INRA standards for maintaining adult weight and for providing adequate nutrition [26]. All animals had free access to water and mineral blocks containing trace elements and vitamins.

2.2. Preparation of females and males

2.2.1. Females

Fig. 1 shows the experimental protocol. Initially the females were in contact with five adult vasectomised males. They were then distributed into six treatment groups that isolated them from males for different periods: 39 days (started February 19th; $N = 15$; Group 39), 30 days (started February 28th; $N = 14$; Group 30), 20 days (started March 10th; $N = 16$; Group 20), 10 days (started March 20th; $N = 14$; Group 10), 5 days (started March 25th; $N = 15$; Group 5), and 0 days (in permanent contact with the vasectomised bucks; $N = 29$; Group 0). These six groups were maintained in shaded open pens under natural day length during the entire experimental period. Both of the used breeds were distributed homogeneously in each group.

2.2.2. Males

Twelve entire males were exposed to 3 months of long days (16 h of light per day) from November 1st, and thereafter to natural photoperiodic conditions. These long days were provided via a mixture of natural light plus artificial light (at least 300 lux at the animals' eye level) from 6:00 to 8:00 h and from 19:00 to 22:00 h. This treatment stimulates testosterone secretion and sexual behaviour in bucks during March and April, i.e., the natural sexual rest period when control males are sexually inactive [20].

2.3. The male effect

On March 30th (Day 0), two males fitted with marking harnesses were placed in contact with each group of females to initiate the male effect and thus start breeding behaviour. The period of breeding lasted 38 days until May 7th.

2.4. Measurements

2.4.1. Detection of oestrous behaviour

During the period of breeding, oestrous activity was recorded by daily visual observation of the marks left by marking harnesses worn by the bucks [27].

2.4.2. Detection of ovulation

To monitor the ovarian cyclicity of the does before their introduction to the males (Day 0; March 30th), blood samples were collected once per week over three consecutive weeks and the plasma progesterone concentration determined. The does were deemed cyclic if their plasma progesterone concentration was >0.5 ng/mL in at least two consecutive samples. This has been shown indicative of ovulation [3,28].

Ovulation was detected, and ovulation rates assessed, via the presence of corpora lutea observed during transrectal ultrasonography performed using an Aloka SSD-500 apparatus connected to a 7.5 MHz linear probe. This was conducted 6–8 days after the detection of oestrus [29]. The presence of corpora lutea was confirmed by the plasma progesterone concentration. Weekly blood samples were taken from the time of introduction to the males until the end of the study. Blood samples were collected by jugular venipuncture in tubes containing heparin. Plasma was obtained by centrifugation at $3500 \times g$ for 30 min and stored at -20 °C until the hormone concentrations were measured.