



Efficiency of the male effect with photostimulated bucks does not depend on their familiarity with goats



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HIGHLIGHTS

- We compared the sexual response of female goats exposed to familiar or novel bucks.
- These males were either sexually active or sexually inactive.
- Sexual active males stimulated sexual response in more goats than sexually inactive.
- Familiarity of sexually active males did not decrease the sexual response of goats.
- Novelty of sexually inactive males did not stimulate the sexual activity of goats.

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ABSTRACT

In ewes, the ovulatory response of females exposed to familiar rams is lower than the response of those exposed to novel ones. In goats, males rendered sexually active by exposure to long days are more efficient to induce ovulation in seasonal anestrus females than untreated males. Two experiments were conducted to determine 1) whether male goats remain familiar to females after 45 days of separation; and 2) whether photostimulated males are able to stimulate the sexual activity of females, independently of their familiarity with them. In **Experiment 1**, three groups of goats ($n = 10$ goats per group) were put in contact with males ($n = 2$ per group) during 10 days in November (familiarization period). These males were called familiar males. After 15, 30 and 45 days of separation from the males, females of each group were exposed to familiar or novel males during 10 min. In each test, goats in contact with novel males displayed more distress bleats, escapes, head butts, and sniffing than those in contact with familiar males ($P < 0.05$). In **Experiment 2**, we used sexually inactive ($n = 4$ control males), and sexually active males ($n = 4$ photostimulated males). In February, two groups of goats ($n = 50$ each) were put in contact with control or photostimulated males ($n = 2$ each) during 10 days ("familiar" control or photostimulated male, respectively). After 45 days of separation from the males, both groups of females were further divided into two groups ($n = 25$ goats per group). In April, two groups were re-exposed to "familiar" control or "familiar" photostimulated males ($n = 2$ per group), whereas the other two groups were exposed to "novel" control or "novel" photostimulated males ($n = 2$ per group). The photostimulated males displayed a higher level of sexual behavior than the controls. The proportion of goats that ovulated and displayed estrus was higher when exposed to the photostimulated males than when exposed to control ones ($\geq 80\%$ vs. 0% ; $P < 0.05$). These proportions did not differ between groups exposed to familiar or novel photostimulated males ($P > 0.05$). We concluded that after 45 days of separation, males are still familiar to females. The photostimulated males are able to induce the sexual activity of seasonally anestrus goats independently of their familiarity with them.

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1. Introduction

In breeds of goats and ewes that display reproductive seasonality, the introduction of a male into a group of seasonal anovulatory females induces an immediate increase in the secretion of LH, leading to

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ovulation associated or not with estrous behavior. This phenomenon is known as the “male effect” [1–3]. Many factors influence the endocrine and ovulatory responses of females exposed to males, among which are the intensity of the male’s sexual behavior, the degree of familiarity with males, and the previous period of separation between sexes.

In small ruminant females showing a strong seasonality, the endocrine and sexual changes associated with the male effect are low or absent in most females during the mid-anestrus, probably due to a decrease of the sexual behavior of males that are also in sexual rest [4–6]. However, when bucks are submitted to artificial long days followed by natural photoperiod to stimulate their sexual behavior during the natural sexual rest, these males become able to stimulate the secretion of LH, estrous behavior and ovulation in most anestrus females [7–9]. These results suggest that the intensity of sexual behavior of males is an important factor for the success of the male effect.

Another factor that could modify the effectiveness of the male effect is the use of “familiar males”, i.e. males already known by females. Indeed, different studies suggest that familiarity plays an important role in social recognition. In fact, it was demonstrated that goats are able to recognize group members relying on olfactory and visual cues [10]. Interestingly, in sheep, females are able to remember familiar individuals over very long periods of time (>2 months; [11]). In addition, Keller et al. [12] demonstrated that ewes were able to recognize familiar flock partners in comparison to a completely unfamiliar female after only 24 h of direct contact. More interestingly, it was shown that the endocrine response of females is higher when they are exposed to novel males in comparison to familiar ones. Indeed, when ewes were exposed for 3 months to males (familiar males), and re-exposed to familiar or novel males after 15 min of separation during the anestrus season, only the novel males were able to increase the frequency of LH pulses [13]. In another study, females remained with males during 3 months, and were then re-exposed to them (familiar males) or exposed to novel males after 15 min or 1 month of separation [14]. This study demonstrated that novel rams increase the pulsatile LH secretion and stimulate LH surge in all females after either 15 min or 1 month of separation, whereas familiar ones are able to increase pulsatile LH secretion only after 1 month of separation and stimulate a preovulatory LH surge in only a third of females [14]. Overall, these results suggest that in ewes, the familiarity of males plays an important role in the response of females to males. In addition, these results suggest that familiar males may become novel again after about 1 month of separation of females.

For decades, it has been thought that females must be previously isolated from males to respond to the male effect. In ewes, it was shown that periods of 21 or 17 days of isolation were sufficient to ensure an ovulatory response in females exposed to males [15,16]. Furthermore, Cohen-Tannoudji and Signoret [17] found that ewes showed an increase of LH pulsatility when they were re-exposed to males after 24 h of separation. Similarly, in goats, it was suggested that a period of at least 3 weeks of separation between sexes was necessary so that females were able to display the male effect [2]. However, it was demonstrated that previous separation between sexes is not necessary when males are naturally sexually active or stimulated by exposure to artificially long days [18,19]. In fact, in one of these studies, most females (9/11) that remained during about three months with sexually inactive males due to the seasonal quiescence, displayed estrous behavior and ovulated when they were re-exposed to photostimulated bucks. In contrast, no females displayed estrous behavior and only 1/11 female ovulated when they were re-exposed to another sexually inactive buck [19]. These later results suggest that the sexual behavior of males plays an important role in inducing the sexual activity of does. However, it is difficult to interpret the results reported by Véliz et al. [19], because the study does not mention if the sexually active or inactive males that were re-introduced with females were familiar or novel bucks.

Considering that photostimulated bucks are very efficient in inducing the sexual activity of seasonally anestrus females, we hypothesized that sexually active bucks would be able to stimulate the estrus and

ovulatory activities of females, independently of their familiarity with them. To test this possibility, we exposed females in seasonal anestrus to a 10-days period of “familiarization” with males that were either subjected to a photoperiodic treatment or kept under natural photoperiod, and re-exposed them to either a familiar or novel male after 45 days of separation.

2. Materials and methods

2.1. General

The procedures used in this experiment were in strict accordance with the Official Mexican Rule for the technical specifications for the production, care, and use of laboratory animals [20].

This study was conducted during the non-breeding season using local goats from Laguna Region at the State of Coahuila, Mexico (latitude 26° 23' N, longitude 104° 47' W). The photoperiod in this region varies from 13 h 41 min of light at the summer solstice to 10 h 19 min of light at the winter solstice. The seasonal anestrus in females isolated from males occurs from February–March to August–September; the sexual rest in bucks isolated from females occurs from December–January to May–June [21,22]. During the whole study, females and males were fed with 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate feed (14% CP; 1.7 Mcal/kg) with free access to water and mineral blocks.

2.2. Experiment 1

2.2.1. Females and familiarization period

On November 15th, female goats were divided in three groups ($n = 10$ each), and kept in three, 10×10 m, different shaded open pens. The distance between pens was at least 100 m. On November 20th, the females of each group were put in direct physical contact with males ($n = 2$) and remained with them during 10 days. The aim of this procedure was that males become “familiar” to females. After the 10-days period of contact, on November 30th, males were removed from the three groups of females and put in another pen located at least 200 m from the pens of females. Females of each group remained together until the recognition tests were performed.

2.2.2. Measurements

With the aim to prove that females are able to recognize males after a previous separation period of 15, 30 and 45 days, we used the paired closed encounters test, which has been previously used to test the discrimination between familiar or novel individuals in sheep [23,12]. Each doe was individually tested in two stages: for half of the females, the test was performed first with a familiar male and second with a novel male. By contrast, the other half of the females was tested first with a novel male and second with a familiar male. The time between the first test and the second test was around 2 h. Before starting the test, each doe was removed from its pen and introduced into an independent pen test (2×2 m) during 2 min. After this period, one “familiar” or “novel” male (no prior contact with females) was introduced during 10 min and the following behaviors displayed by the doe were scored; the number of distress bleats, escapes, head butts, and sniffing. After the tests, each doe was placed in a new independent pen with the aim of avoiding the transmission of smell of the male to the other females.

2.2.3. Statistical analyses

The Wilcoxon Signed-Rank test was used to compare the behaviors displayed by each female in presence of familiar or novel males within each test. The Kruskal-Wallis test was used to compare the behaviors displayed by females in presence of familiar or novel males between each test.

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