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# No previous isolation of female goats is required for novel males to induce a male effect, especially if direct physical contact is established

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ABSTRACT

Goat does supposedly need to be separated from bucks before male stimuli can induce reproductive activity, ovulation, and estrous. The present study examined the reproductive response (ovulation and estrous) of does to "novel" bucks in direct contact with them and when separated from them by a fence. One hundred fourteen does were distributed into three groups: (1) ISOL group (N = 31): females that had been completely isolated from males for 46 days, and thereafter placed in direct contact with "novel" males during the breeding period; (2) NOTISOL-CONTACT group (N = 29): females that had been in contact with "familiar" vasectomized males for 46 days and thereafter placed in direct contact with novel males during the breeding period; (3) NOTISOL-NO CONTACT group (N = 54): females that were in contact with familiar vasectomized males for 46 days and thereafter introduced to novel males, but separated by a fence, during the breeding period. All the males were treated with melatonin to ensure that they were sexually active. Estrous 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 (measured in weekly blood samples). The ovulation rate was assessed by transrectal ultrasonography. Fecundity, fertility, prolificacy, and productivity were also determined for the ISOL and NOTISOL-CONTACT groups (naturally, in the NOTISOL-NO CONTACT group, no pregnancies were possible). The introduction of novel males induced the same ovarian response in each group ($P > 0.05$), but the percentage of females that showed estrous and ovulation was lower in the NOTISOL-NO CONTACT group ($P < 0.05$). No differences were seen between the ISOL and NOTISOL-CONTACT groups ($P > 0.05$) in terms of fecundity, fertility, prolificacy, and productivity. These results show that the introduction of novel males to females already in contact with familiar males induces ovarian activity similar to that observed in the classical male effect (ISOL group) and that this response leads to a significantly higher estrus activity only when direct physical contact between the females and the novel males is established.


## 1. Introduction

Seasonal reproduction in the goat can be modified *via* the establishment of sociosexual relationships. In fact, the

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sudden exposure of anovulatory goats to males results in a rapid increase in LH pulse frequency, followed by a pre-ovulatory LH surge and ovulation (see reviews by Chemineau [1] and Delgadillo et al. [2]). This phenomenon has been extensively studied in goats [2–4]. Factors such as previous isolation from males, male novelty, continuous contact between the sexes after male introduction, and the intensity of male sexual behavior may all influence the response of females [2].

Traditionally, it has been assumed that females had to be isolated from male stimuli if the male effect was to induce ovulation. However, the introduction of a "novel" ram can stimulate the endocrine and ovulatory activities of ewes maintained in full contact with males just as efficiently as in ewes isolated from all rams [5,6]. In goats living at Mediterranean latitudes, Zarazaga et al. [7,8] observed no estrous response during seasonal anestrous in female goats that were permanently in contact with sexually active males probably because these males were considered as familiar to them. It would seem that females have the ability to identify and remember individual male signals and therefore recognize familiar and novel rams. Some authors suggest that this helps explain the ability of novel males to induce ovulation in ewes [5,6]. However, the importance of the introduction of novel males in goats has not been studied.

It was traditionally assumed that, once males had been introduced to females, permanent contact between the sexes was required to maximize female endocrine and ovulatory responses to male stimuli [9–11]. However, in Angora goats, it was shown that the odor of a buck on its own was enough to induce ovulation [12]—the effect of an olfactory molecule now known to activate the central reproductive axis in female goats [13]. However, the percentage of goats that ovulate is lower than that when direct contact with the bucks occurs [12], suggesting that physical contact is required to obtain a high percentage response. Similarly, in French Saanen does, indirect contact with males established using an uncrossable corridor leads to a reduction in the percentage of ovulating females compared with that seen when the sexes are in direct contact [1].

A key influence on the response of females to males is the sexual behavior, the latter display. In ewes, a greater proportion of females ovulate when they are exposed to males that were well able to service them than when males are of lesser capacity [14]. In goats, all females ovulated after exposure to males rendered sexually active during anestrus *via* exposure to long days followed by the natural photoperiod; in contrast, no female ovulated when exposed to nontreated males that were also in their period of sexual rest [15].

It was hypothesized that, at Mediterranean latitudes, does would respond to novel males without the need for previous separation from familiar males, even when no direct physical contact with the novel males was allowed. The aims of the present study were therefore to determine (1) whether the introduction of novel bucks to does in permanent contact with males induces female reproductive activity and (2) whether any similar response occurs when no direct physical contact is allowed.

## 2. Materials and methods

### 2.1. 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).

### 2.2. Animals and management

The females used in this work were adult (2–3 years old), nonpregnant, Blanca Andaluza does (N = 114). These were assigned to two initial groups: females in permanent contact with six males (initial contact group, n = 83 does) and females isolated from males (ISOL group, n = 31 does). Over the experimental period, these females were maintained indoors and fed daily with lucerne hay, barley straw, and commercial concentrate, according to Institut National de la Recherche Agronomique standards for maintaining adult weight and for providing adequate nutrition [16]. All animals had free access to water and mineral blocks containing trace elements and vitamins.

To determine their ovarian cyclicity before the introduction to the males (Day 0; May 25), blood samples were collected once a week over three consecutive weeks to determine the plasma progesterone concentration. The does were deemed cyclic if their progesterone concentration was greater than 0.5 ng/mL in at least two consecutive samples. This has been shown indicative of ovulation in Payoya goats [17,18].

### 2.3. Preparation of females and males

#### 2.3.1. Females

Figure 1 shows the experimental protocol. On April 9, females in the ISOL group were isolated from males for a period of 46 days. The does in the initial contact group remained in contact with six vasectomized males (hereinafter referred to as familiar males) fitted with marking harnesses during this same period.

#### 2.3.2. Males

On April 9, eight bucks of Blanca Andaluza breed received two subcutaneous melatonin implants containing 18 mg of the hormone (Melovine, CEVA Salud Animal, Barcelona, Spain) at the base of the left ear to stimulate their libido during the sexual rest period [7,19,20]. These melatonin-treated males are hereafter referred to as "novel" males. All males in direct contact with females wore marking harnesses. All familiar males were vasectomized, and all novel males were entire.

### 2.4. The male effect

On May 25 (Day 0), two novel males were put in contact with the ISOL group of females. At this moment, the group