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Sexually active bucks are able to stimulate three successive groups of females per day with a 4-hour period of contact

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ABSTRACT

Bucks rendered sexually active by a photoperiod treatment of long days can induce fertile ovulation in a group of goats with only 4 h of contact daily with a male:female ratio of 1:10. Here we tested whether such bucks could induce fertile ovulations when stimulating successively three different groups of anovulatory goats when interacting 4 h per day during 15 consecutive days. Control males ($n=3$) were introduced in the control group ($n=25$) of does at 8:00 h and were removed at 12:00 h. Experimental males ($n=3$) were in contact with the experimental groups of does: from 8:00 h to 12:00 h with a first group ($n=27$), from 12:00 h to 16:00 h with a second group ($n=26$) and with a third one ($n=27$) from 16:00 h to 20:00 h. Bucks were then placed until next day in another pen. Both in the control and the experimental groups, more than 85% of females ovulated, and the proportions did not differ between the control and experimental groups ($P\geq 0.67$) or between the three experimental groups ($P\geq 0.67$). Moreover, the ovulation rate did not differ significantly between the control and the experimental females nor between the three experimental groups. Bucks were able to fertilize more than 72% of does independently of the number of females they were exposed to ($P\geq 0.17$). Finally, more than 58% of females kidded and fertility did not differ between the control and experimental groups ($P=1$) nor among experimental groups ($P\geq 0.77$). We conclude that sexually active bucks are able to induce fertile ovulation in three successive groups of anovulatory goats even when the period of contact between sexes is reduced to 4 h per day.

1. Introduction

In breeds of sheep and goats that show a period of seasonal anestrous, the introduction of a male into a group of anovulatory females can stimulate their sexual activity within 72 h. This phenomenon, known as the "male effect", has been extensively studied in ewes [1–3] and goats [4–6]. Factors such as the duration of contact between sexes, the intensity of the males' sexual behavior and the male:female ratio may influence the response of females exposed to males [6].

In ewes, it has been shown that the presence of the male 24 h per day during 15 days is required to obtain a maximum ovulatory response [7]. However, recent studies indicate that the decrease of the duration of contact between sexes from 24 to 16, 12, 8 or 4 h per day still enables to induce a high ovulatory response and fertility in Mexican goats [8,9]. A likely explanation for the difference between the studies in ewes [7] and goats [8,9] is that long-days-treated – and therefore sexually active – bucks were used in these two latter studies, whereas in the first one, they used rams that had not been treated with light and which were therefore probably either in sexual rest or with a low sexual behavior. Indeed, it was found in both goats and ewes, that a high sexual activity of males is a key component to obtain a high ovulatory response of females to the male effect [10,11].

A recent study in goats indicates that the females' estrous response is not affected by decreasing male:female ratio but that some specific variables are [12]. Indeed, Carrillo et al. [12] found that a decrease in the male:female ratio from 4:39 to 2:39 or 1:39 had no effect on the occurrence of estrous behavior but lengthened mean interval between introduction of males and onset of estrous behavior. In our breeding conditions, several experiments have shown that a male:female ratio from 1:8 to 1:13 [10,13–15] ensures proper fertilization of females and, more recently, it was demonstrated that such a ratio is sufficient to induce high ovulatory and reproductive responses in females even when daily contact between the buck and the females is reduced to 4 h [9]. However, whether sexually active bucks are able to induce ovulation, sexual activity and adequate fertilization to three groups of anestrous goats stimulated successively when the daily period of contact is 4 h remains to be investigated. Considering that a male:female ratio of 1:10 enable high ovulatory and reproductive responses,

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it could be expected that sexually active bucks will be able to stimulate three groups of females when the ratio is in this range and the daily period of contact is 4 h. To test this possibility, we exposed daily sexually active bucks either to a single or to three different groups of females during 4 h, at a ratio of 1:9 and for a period of 15 consecutive days.

2. Materials and methods

2.1. Conditions of the study and females

The experiment was performed using local goats (*Capra hircus*) from the Laguna region in the State of Coahuila, Mexico (latitude, 26°23′N and longitude, 104°47′W). In females, non-breeding season lasts from March to August, and in bucks from January to April [16,17]. All females were multiparous and had given birth between October and December and were milked manually once daily during the study. Females and males were fed 2 kg of alfalfa hay (18% CP) and 200 g of commercial concentrate (14% CP; 1.7 Mcal/kg) with free access to water during the study.

All the females used were anovulatory at the beginning of the experiment. To ensure this, on March 15th and 23th, each female was submitted to a transrectal ultrasonography using an Aloka SSD-500 machine connected to a transrectal 7.5 MHz linear probe in order to determine their ovarian cyclicity. This method was previously described by Ginther and Kot [18] and proved to be reliable for the assessment of luteal activity in goats [19]. On March 24th, anovulatory females were divided into four groups balanced for live weight and body condition score and kept in open pens (6×4 m; Table 1). Body condition score was assessed by palpating the spinous and lateral processes, and the musculature of the lumbar region of the spine and allocating a score from 1 (very lean) to 4 (fat) in increments of 0.5 [20].

2.2. Sexual activation of bucks – photoperiodic treatment, semen quality and sexual behavior

Six bucks were kept together in an open pen (6×6 m). The males were subjected to a treatment of long days (16 h of light/8 h of darkness) from November 1st to January 15th. On January 16th, the light treatment was stopped and the bucks were exposed to natural variations of day-length until the end of the study. This treatment has previously been shown to take a further 45 to 60 days to produce a stimulatory effect on male reproductive activity [10]. Testosterone secretion is stimulated from the end of February to the end of April and, as a consequence, the production of male odor and the sexual behavior of bucks are improved during these months corresponding normally to the non-breeding season [8,10]. For this reason, light-treated males are used in March–April. On March 17th, the quality of semen was assessed in undiluted ejaculates by determining the progressive sperm motility and the percentage of live spermatozoa observed immediately after semen collection [21]. Semen was collected using an artificial vagina, when males were presented to an intact estrus-induced doe. All males had good quality semen with more than 70% of live spermatozoa and sperm motility greater than three (0: no movements; 5: rapid and rectilinear movements [21]). On March 22nd, bucks were individually submitted to behavioral tests in order to confirm their high sexual behavior before beginning the experiment. Every buck was exposed to an anestrous goat and the following sexual behaviors were recorded: self urination, ano-genital sniffing, nudging, mounting attempts and mounts with vaginal intromission [22,23]. From the 6 bucks rendered sexually active by the light treatment, two groups ($n=3$ each) balanced for semen quality and sexual behavior were formed: a group of control males and a group of experimental males.

Table 1
Characteristics of the goats of the control group (C) that were exposed to three males from 8:00 h to 12:00 h and the experimental groups that were exposed to three other males from 8:00 h to 12:00 h (G8–12), from 12:00 h to 16:00 h (G12–16) and from 16:00 h to 20:00 h (G16–20). Each group of females was in contact with males 4 h per day during 15 days. Males were rendered sexually active by exposure to long days (16 h of light by day) from November 1st to January 15th.

Groups	*n*	Live weight kg (Mean ± SEM)	BCS[a] (Mean ± SEM)
C	25	42 ± 1	1.9 ± 0.1
G8–12	27	41 ± 1	1.9 ± 0.1
G12–16	26	41 ± 1	1.9 ± 0.1
G16–20	27	41 ± 1	1.9 ± 0.1

[a] BCS = body condition score (range 1 to 4).

2.3. Male effect

On March 27th (day 0), females were exposed to bucks for 15 days. Control males were in contact with one group of females from 8:00 h to 12:00 h (C). Experimental males were in contact with three successive groups of females: with the first group from 8:00 h to 12:00 h (G8–12); with the second group from 12:00 h to 16:00 h (G12–16) and with the third group from 16:00 h to 20:00 h (G16–20). Each group was divided into three sub-groups so that each buck stimulated individually 8 or 9 females. After the daily period of contact, bucks were placed together until the next day in another pen located at more than 200 m from the females' pens. In addition, the distance between the four groups of females was more than 100 m, thus preventing any risk of interference by the treatments between groups or sub-groups [24]. Females remained in the pens of stimulation during the whole experiment. The experimental protocol is showed in Fig. 1.

2.4. Measurements

2.4.1. Females

The male-induced ovulation and ovulation rate were assessed by the presence and number of corpora lutea, respectively, observed in each female by transrectal ultrasonography with the same equipment as above, 20 days after introduction of the bucks [18,19]. Pregnancy rate (pregnant does/does exposed to males) was determined by abdominal ultrasonography 60 days after exposure to males using the same machine connected to a 3.5 MHz abdominal probe. Fertility (number of females kidding/number of females exposed to males) and prolificacy (number of kids born/number of females giving birth) were determined at parturition.

2.4.2. Males

Sexual behavior of the bucks was observed on days 1, 2, 7 and 8 after their introduction into the groups of females. Each day, the observations were carried out during the first hour of contact between sexes in each group of females i.e. from 8:00 h to 9:00 h in C and G8–12; from 12:00 h to 13:00 h in G12–16 and from 16:00 h to 17:00 h in G16–20. Trained observers followed bucks individually and recorded the following behaviors: self urination, ano-genital sniffing, nudging, mounting attempts and mounts with vaginal intromission [22,23].

2.5. Statistical analyses

The Chi-square test was used for multiple-group comparisons and the Fisher's exact probability test was used for two-group comparisons to assess statistical differences in the proportions of females that ovulated, pregnancy rates and fertility. Ovulation rate and prolificacy were all compared using the Kruskall–Wallis-test followed by the Mann–Whitney *U* test. Males' behavioral components were analyzed with a Chi-square test for goodness of fit considering a random distribution of 50% in each group when comparing control and experimental