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Male effect in seasonally anovulatory lactating goats depends on the presence of sexually active bucks, but not estrous females

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Abstract

A study was conducted in subtropical northern Mexico (26°N) to determine whether the presence of estrous females can improve the response of seasonally anovulatory goats to the introduction of bucks in the group. The induction of estrous activity was studied in three groups of anovulatory lactating goats during seasonal anestrus. These females were of the

Mexican Creole breed. In the control group (sexually inactive (SI), n=20), two control (SI) bucks exposed to normal seasonal daylength variations were used. In the second group (SI+E, n=20+3), two control males were also used, but in addition, three females of the group were in estrus at the time of male introduction. In the third group (sexually active, SA+E, n=19+4), anovulatory females were exposed to two bucks made sexually active by exposure to 2.5 months of long days (16L:8D) followed by two subcutaneous 18 mg melatonin implants, and four estrous females were also present when introducing the bucks. In all groups, males were introduced on 15 March and estrous detection was conducted twice daily for 15 days. The sexual activity of the bucks was observed from 08:00 to 10:00 h during the first five days of exposure to females. More females displayed estrous behavior in the first 15 days following the introduction of the males in the SA+E group (18/19) as compared with the SI or SI+E groups (2/20 and 0/20, respectively; $P < 0.001$).

No difference was observed between the two latter groups. Thirteen females of SA+E group showed a second estrus between days 6 and 11 (short estrous cycle duration: 5.4 ± 0.4 days). By contrast, in the SI group none showed a second estrus. The sexual behavior of the males in the SA+E group was greater as compared with that of the males in SI and SI+E groups (over 80% of the total sexual activity recorded in the three groups; $P < 0.001$). By contrast, no differences were found between SI and SI+E males. These results indicate that the presence of estrous females alone at the time of buck introduction is not sufficient to induce an adequate stimulation of seasonally inactive males. The use of sexually active bucks is necessary to induce reproductive activity in anovulatory females, whereas preparation of the bucks with long days followed by melatonin implants allows them to gain such a capacity.

Author Keywords: Goat; Reproductive seasonality; Photoperiod; Estrus; Male effect; Sexual behavior

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1. Introduction

While reproductive activity in mammals is dependent on hormonal factors, in several instances the social environment can exert some modulatory action (Malpaux and Gerlach). Thus, in seasonal breeds of sheep and goats, sexual activity can be induced by the introduction of a male into a group of seasonally and/or lactationally anovulatory females male effect (ME) or teasing; ewes (Underwood; Poindron and Signoret); goats (Shelton and Chemineau), and the amplitude of the response to male exposure depends on several factors (Walkden-Brown et al., 1999). For example, the percentage of lactationally anestrous females that respond to the male effect varies with the amount of sexual behavior displayed by the males. In a study conducted by Perkins and Fitzgerald (1994), it was found that 97% of ewes ovulated following the introduction of males showing an intense sexual behavior, while only 78% of females did so in response to stimulation by males with a lesser sexual behavior. Also, the proportion of females ovulating in response to exposure to a male is greater when both sexes can interact physically as compared with when physical contact is prevented by a fence line that does not block visual contact (goats (Chemineau, 1987); ewes (Pearce and Oldham, 1988). Another factor that can influence the response to ME is the presence of females in estrus at the time of male exposure to females. Thus, exposing rams (Rosa et al., 2000) or bucks (Walkden-Brown et al., 1993b) to estrous females shortly before or at the time of introduction of the males into a group of anovulatory females, increases the response induced in these anovulatory females. In a study by Walkden-Brown et al. (1993b), 87% of goats responded to the male effect when estrous females were present at the time of male introduction versus 72% in their absence. In fact, the presence of only a few estrous ewes or goats may induce sexual activity in the rest of the females, even in the absence of any male (ewes (Zarco et al., 1995); goats (Bouillon; Restall and Alvarez). In subtropical northern Mexico, it has been recently reported that seasonally anovulatory lactating female goats only respond to the male effect if sexually active males are used. Indeed, in a study performed in mid-March, about 80% of Mexican Creole goats showed at least one bout of estrous behavior within 14 days after the introduction of bucks in which sexual activity had been stimulated by a long-days+melatonin treatment, while virtually no response was found in females exposed to control males that were sexually inactive (Flores et al., 2000).

However, it has not been determined if the response of anovulatory females to sexually inactive males can be improved by exposing those males to estrous females at the time of male introduction. The hypothesis of the present study was that in a group of seasonally anovulatory goats estrous response to the introduction of sexually inactive bucks would be greater if some females were in estrus when the bucks were introduced as compared to when no females were in estrus at that time. A third group was also studied, in which sexually active bucks were used to have a positive control group, to make sure that, if no response was observed in either of the two groups with inactive bucks, it was not due to the inability of the females to respond, independently of the type of males used. In the three groups sexual behavior of the males was measured because the hypothesis implied that the response of the females in a given group would also be related with the sexual activity of the males in that group.

2. Materials and methods

2.1. Animals, maintenance conditions and treatments

2.1.1. Males

Ten adult Mexican Creole male goats of the Laguna region in the State of Coahuila, Mexico (26°N) were used. The characteristics of these bucks were the same as those described previously by Delgadillo et al. (1999) and used by Flores et al. (2000). All animals were kept outdoors and fed alfalfa (18% CP) ad libitum, and 300 g of commercial concentrate (14% CP) and free access to water and mineral blocks. The preparation of the males started on 1 November 1999. One group (sexually inactive (SI); n=5) was exposed to natural photoperiodic variations throughout the study (13 h and 41 min of light at the summer solstice and 10 h and 19 min at the winter solstice). The other group (sexually active (SA); n=5) was subjected to a treatment of long days (16 h of light/8 h of darkness) from 1 November 1999 to 15 January 2000. On 16 January, each SA male received two subcutaneous implants containing 18 mg of melatonin in each implant (Regulin-Melovine, CEVA Sant Animale, Libourne, France). From this day onwards, the light treatment was stopped and the males were exposed to natural day length variations until the end of the study. This treatment has been shown previously to stimulate LH and testosterone secretion and, as a consequence, to improve sexual behavior of bucks during the non-breeding season (Flores and Delgadillo).

2.1.2. Females

One hundred and two multiparous Mexican Creole goats were used. They had given birth between 1 October 1999 and 15 February 2000. All females were hand milked once a day during the time the experiment was conducted, and none of them were rearing kids during the experimental period. Kids had been weaned at about 25 days of age. The females were not allowed with males from 1 January 2000, and the nearest bucks were in another flock situated about 2 km away from the experimental farm. The females were maintained under extensive management system before the study was started. On 23 February, the goats were penned in

a yard and fed alfalfa (18% CP) ad libitum, and 200 g of commercial concentrate (14% CP), with free access to water and mineral blocks until the end of the study. On 24 February and 5 March, blood samples were taken by veinipuncture and plasma progesterone concentrations determined by RIA, a procedure which allows for distinguishing females which have initiated estrous cycles from those which remain anovulatory (Terqui and Thimonier, 1974). Thirty-four does showed ovarian activity and were, therefore, separated from the main flock and removed from the experiment. As a consequence, only 68 females were used in the experiment.

2.1.3. Male effect

On 14 March 2000, females were divided in three homogeneous groups balanced for parturition date (month), body weight and amount of milk production. The distance between each group was more than 130 m (Walkden-Brown et al., 1993b).

The control group (SI group) consisted of 20 anovulatory females that were exposed on 15 March to two control bucks in non-breeding season selected at random from the SI group described previously. The second group (SI+E group) consisted of 24 anovulatory females, of which 20 remained untreated, while the remaining four were synchronized to be in estrus on the day of exposure to bucks (see details of synchronization technique below). These females were exposed to two randomly selected SI bucks as in the first group. In the third group (SA+E group), 24 anovulatory females were used. Twenty were left untreated and the four remaining ones were synchronized into estrus as in the SI+E group. On the day of buck exposure (day 0), this group was exposed to two sexually active bucks selected at random from the SA group of bucks. Finally, an additional blood sample was collected on the day of buck exposure from all females of the three groups, to check that no females other than the ones induced into estrus had initiated ovarian activity at this time.

2.1.4. Estrous induction in stimulus females

Females to come into estrus on the day of introducing the males were treated with vaginal sponges impregnated with 45 mg of a progestagen for 10 days (FGA, Chronogest, Intervet), and 48 h before the sponge removal, the animals were injected with, 200 IU of eCG i.m. (Folligon, Intervet, Holland) and 250 g of prostaglandin PGF₂ i.m. (Lutalyse, Upjohn, Mexico; Leboeuf et al., 1998). Four females were treated in each of the SI+E and SA+E groups. Of these, three in the SI+E and four in the SA+E groups were in estrus at the time of male exposure to females. They remained in their respective group until the end of the study, on 30 March 2000.

2.2. Measurements

The males were introduced into their respective group on 15 March 2000 at 08:00 h. In each group, the sexual behavior of the bucks was observed for two consecutive hours, from 08:00 to 10:00 h during the first five days of the male exposure period. Trained observers followed bucks individually and recorded the following behaviors: ano-genital sniffing, nudging, mounting attempts, mounts (without and with ejaculation) and self-urination (Flores and Walkden). For the females, in addition to acceptance of the male during the daily observations up to day 5, the presence of estrous behavior was checked twice daily from 15 to 30 March. To

accomplish this, the bucks used for male exposure were permanently fitted with marking harnesses and does were inspected at 08:00 and 18:00 h for recording of new raddle marks (Radford and Walkden).

2.3. Statistical analyses

Because the data consisted of frequencies of occurrence, non-parametric statistics were used (Siegel and Castellan, 1994). The total proportions of anovulatory females coming into estrus were compared using Fisher exact probabilities. Differences in the frequencies of male sexual behaviors recorded in each group were analyzed using χ^2 for goodness of fit or a Binomial test, depending on sample size and presence of low frequencies (Siegel and Castellan, 1994). The frequencies observed in each group were compared against the null hypothesis that behaviors would be equally distributed in the three groups, that is the expected frequency of a given behavior in any group would be one-third of the total observed frequency for this behavior. Also the frequencies of sexual behavior were compared between any two groups, using a Binomial test with a theoretical expected probability of 0.5 in each group.

3. Results

3.1. Response of females to the male effect

One female of the SA+E group showed health problems at the beginning of the study, therefore, data from this female were excluded from the analyses. The proportion of females that responded to the male effect by showing at least one behavioral estrus during the first 15 days following the introduction of the bucks was significantly greater in the SA+E group as compared with the SI or SI+E groups (18/19 versus 2/20 and 0/20, respectively; Fishers exact $P < 0.001$ in both cases). No significant difference was found between the latter two groups.

In the SA+E group, 15 of 19 does displayed estrous behavior within the first three days after male introduction and 3/19 did so 8 and 9 days later. The interval between the introduction of males and the first estrous behavior was 3.0 ± 0.6 days (Fig. 1), while 13/18 females showed a second estrus between days 6 and 11 (short estrous cycle duration: 5.4 ± 0.4 days). By contrast, in the SI group neither of the two goats that came in estrus 3 and 4 days after introduction of males showed a second estrous behavior within 15 days.

(9K)

Fig. 1. Proportion of seasonally anovulatory goats responding to a male effect with estrous activity being displayed. Goats showed estrous behavior in a group of females that did not contain estrous females at the time of introduction of sexually inactive (SI) bucks (a); no goats showed estrous behavior in a group of females that contained estrous females at the time of introduction of sexually inactive (SI+E) bucks (b); goats showed estrous behavior in a group of females that contained estrous females at the time of introduction of sexually active (SA+E) bucks (c); day 0 is day of buck exposure.

3.2. Sexual behavior of bucks

The frequency of manifestation of all sexual behaviors during the first five days following the introduction of the males differed significantly between groups. For all behaviors, the majority of them (80% or more; Fig. 2) was expressed in the SA+E group (489 ano-genital sniffing, 758 nudging, 14 mounting attempts, 16 mounts and 8 self-urination). This was significantly greater than a random distribution ($P < 0.001$ in all cases, 2 or Binomial test), and greater than in any of the two other groups (Binomial test, $P < 0.001$), whereas no differences were found between SI and SI+E groups (Binomial test, $P > 0.05$).

(10K)

Fig. 2. Sexual behavior of bucks following their introduction in a group of anovulatory females. For each variable, results are expressed as the percentage of behavior displayed in each group, relative to the total number of behaviors observed in the three groups during the first five days after buck exposure and with 2 h of observation daily per group (9 self-urination, 526 ano-genital sniffing, 769 nudging, 14 mounting attempts, and 18 mounts, total number, respectively). Shaded bars: sexually inactive bucks introduced in a group of 20 anovulatory females (SI). Open bars: sexually inactive bucks introduced in a group of 20 anovulatory females also containing three estrous females (SI+E). Solid bars: sexually active males introduced in a group of 20 anovulatory females also containing four estrous females. For each variable, between groups values with different letters are significantly different (Binomial test, $P < 0.05$).

4. Discussion

The results of the present study fail to support our initial hypothesis that the presence of estrous females in a group of anovulatory goats would facilitate their response to the male effect. Indeed, the presence of estrous-induced females did not result in a significant response in anovulatory lactating Creole goats to the male effect using sexually inactive males under to the natural photoperiod variations (SI): only two females of the SI group and none of the SI+E group showed estrous behavior. On the contrary, the females in contact with SA males displayed a clear activation of behavioral reproductive activity, indicating that the lack of response in the other two groups was not due to some inherent inability of these females to respond to buck exposure with an induced ovulation and initiation of estrous cycles. However, a greater percentage (68%) of females of the SA+E group initiated estrous cycles of short duration as compared with the other groups. This physiological response is similar to the estrous cycle duration reported by others in sheep and goats (Walkden and Walkden; Chemineau et al., 1993). The mechanisms of early luteolysis are probably due to the early regression of corpora lutea induced by the male effect in anoestrous females. However, it appears that the "quality" of preovulatory follicles could be one of the major factors regulating the length of induced estrous cycles (Chemineau and Lassoued).

Concerning the behavior of the males, the non-treated bucks showed lesser amounts of sexual behavior when introduced with the females, and the presence of receptive goats in the group had no effect in this respect. This is in agreement with the premise that exposure to estrous females does not result in immediate stimulation of LH and testosterone secretion and sexual behavior in bucks outside of the breeding season (Walkden-Brown et al., 1994). Bucks submitted to photoperiod plus melatonin treatment did, however, have greater sexual behavior.

This is consistent with the results of previous studies using similar photoperiodic treatments to induce out of season reproductive activity in bucks (Delgadillo and Flores). The results from the present study are not consistent with those of Rosa et al. (2000) in sheep and of Restall et al. (1995) and Álvarez-Ramirez et al. (1999) in goats. Contrary to these earlier findings, we found no evidence that the presence of estrous females in the group facilitated, either directly or indirectly, the sexual behavior of the bucks, or the activation of the reproductive activity of anovulatory does.

Several factors may account for these differences. The time of the non-breeding season at which buck exposure is performed (Restall and Mellado), the fact that females were lactating as well as in seasonal anestrus in previous studies (McNeilly and Flores), and the level of nutrition before male exposure (Wright et al., 1990), are known to influence the response of the females to ME, both in sheep and goats. Some of these factors may have influenced the ability of the females to respond to bucks by initiating ovulatory cycles in the present experiment, and explain that no effect was observed when seasonally anovulatory goats were exposed to sexually inactive bucks during non-breeding season, even in the presence of estrous females. However, and regardless of the possible contribution of these factors to the absence of response in the SI and SI+E groups, it remains that females of the SA+E group responded to buck exposure (95%), despite the fact that all females were from the same flock and management conditions. This indicates (a) that females were able to respond if adequately stimulated by bucks and (b) that the difference of response between treatments cannot be attributed to pre-experimental differences between the groups of females. The response of anovulatory females exposed to SA males was not studied in the present study. Nevertheless, it is known from previous experiments conducted under similar conditions (Flores and Delgadillo), that such females are fully able to respond to SA males (93%), even in the absence of estrous females in the group at the time of buck exposure. Therefore, it appears that the photoperiodic+melatonin treatments applied to the males prior to their introduction was a key element of successful induction of onset of estrous cycles in seasonally anovulatory and lactating goats. At these times, females did not respond to sexually inactive males. One clear effect of the treatment was to induce a great amount of sexual activity in the bucks, all components of sexual behavior being significantly greater as compared with the two other groups, and male sexual behavior is known to be one of the factors that induces ovarian activity in anestrus ewes and goats (Cohen and Flores). Therefore, this is certainly one of the ways by which treatment of the males induces the response in the females. In addition, this treatment also activates the physiological events normally occurring during the breeding season in the male (Lincoln and Delgadillo). In turn, this probably results in an increase in the production of olfactory signals by the male, which are also known to have a role in the male effect, even though they only induce ovulation in about 40% of females versus 95% when a

buck is used (Walkden-Brown et al., 1993a). At this stage, however, it is not clear whether male sexual behavior by itself is a sufficient stimulus to induce the response in the conditions of the present study, or if the combination of behavior and olfactory signals is needed to obtain response.

5. Conclusion

We can conclude from results of the present study and those of Flores et al. (2000) that a photoperiodic treatment of long days and melatonin is effective in ensuring the success of the male effect in seasonally anestrus females.

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