

## Ram sexual preferences and estrous behavior expression in ewes with different reproductive status

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**Abstract:** The hypothesis was that the level of sexual experience and reproductive status of ewes affect the time that rams dedicate to courtship. Ewes (n = 40) were separated into four groups according to their reproductive status: maiden, empty, under continuous and restricted suckling ewes. Rams (n = 8) were separated into four groups according to their sexual experience: experienced-I and -II and inexperienced-I and -II. Each group of ewes was exposed at the same time to a different group of rams at 10–12 h intervals for 22 days, an injection of prostaglandin was given to ewes at the end of this period. The response variables were the time that each group of rams spent courting each group of ewes during the first 22 days of the experiment and the number of ewes from each group that showed estrous behavior during and after the 22 days of the experiment. Sexually inexperienced rams spent more time courting maiden ewes than any other group of ewes ( $p \leq 0.05$ ) ( $39.59 \pm 3.01$ ,  $29.41 \pm 3.01$ ,  $26.31 \pm 3.01$  and  $23.36 \pm 3.01$  minutes for the maiden, continuous, restricted and empty groups, respectively). Experienced rams dedicated more time to courting the group of empty ewes ( $p \leq 0.05$ ) ( $13.55 \pm 3.01$ ,  $17.68 \pm 3.01$ ,  $14.9 \pm 3.01$  and  $20.05 \pm 3.01$  minutes for the maiden, continuous, restricted and empty groups, respectively). Expression of estrous behavior was affected ( $p \leq 0.05$ ) by the reproductive status of the ewes during the first 22 days of the experiment; the higher difference was in favor of ewes from the empty group. In addition, a higher display of estrous behavior was observed after prostaglandin injection ( $p \leq 0.05$ ). In conclusion, the level of sexual experience and the reproductive status of the ewes affects the time that rams spend in courtship.

**Key words:** Sexual behavior, ram courtship, mating behavior, courting behavior

### 1. Introduction

Knowledge and understanding of domestic animal behavior have contributed to domestication and management [1], and sexual behavior is not an exception. Sexual behavior is characteristic in every species, involving a series of events such as courtship and copulation [2]. Sexual preference is part of the sexual behavior that stimulates seeking out and choosing a partner to mate [3]. Such preferences are imprinted in the early stages of sheep life [4], but not the sexual behavior itself, which is fully developed only after repeated sexual encounters [5, 6].

Even though sex preferences are established early in life, rams and ewes are more attracted to certain types of animals. Ewe sexual preferences and behavior are affected by ram physical conformation [7], male sexual performance [8], and by sheep temperament [9]. They usually initiate

the sexual encounter by seeking out the ram during estrus [10], which increases ram sexual activity [11]. On the other hand, rams display more courtship and mating behavior toward tailed than toward docked ewes [12], and they also prefer to mate with ewes that spontaneously show estrus than with those with synchronized estrus [13]. In addition, the presence of more than one ewe in estrus or male competitors are factors that are known to significantly affect sexual behavior in rams [14].

The reproductive performance of a ram is affected by several factors, such as body condition score, testicle size, age, libido, among others [15, 16]. It is important to bear these factors in mind, as well as male sexual preferences, to maximize ram reproductive performance. The latter is important in the context of the “male effect” consisting of sudden introduction of the ram to ewes.

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This biostimulation strategy has been successfully used to induce ovulation in females during seasonal anestrus [17], but its effect depends on the intensity of male sexual behavior manifested by courtship activities, including nudging, anogenital sniffing, mounting attempts or performing flehmen [18]. A more intense courtship of rams exposed to ewes they feel more attracted to can be expected than of those exposed to less attractive ewes. This might explain the contradictory results regarding the “male effect” on reproductive variables measured in ewes with different reproductive status [19, 20]. We suspect that sexual reproductive status of the female affects the time that the ram spends displaying sexual behavior.

Thus, we hypothesized that the level of sexual experience and the reproductive status of ewes affect the time that rams dedicate to courtship.

## 2. Materials and methods

### 2.1. Animals and experimental procedure

The ewes ( $n = 40$ , crossbred Dorper  $\times$  Pelibuey  $\times$  Katahdin) were separated into four different groups according to their reproductive status: maiden ewe lambs ( $n = 10$ ), empty ewes ( $n = 10$ ), ewes under continuous suckling ( $n = 10$ ), and ewes under restricted suckling ( $n = 10$ ). The maiden ewe lambs were 7 months old with an average body weight of  $25.2 \pm 0.9$  kg regardless of whether they had reached puberty at the beginning of the study was unknown. The empty ewes were  $\geq 2$  years old, nonpregnant, not with a lamb, and with body weight of  $48.3 \pm 1.5$  kg. The ewes under continuous suckling were  $\geq 2$  years old weighing on average  $48.3 \pm 1.3$  kg and  $17 \pm 1.9$  days postpartum. The ewes in this group and their lambs remained together during the entire study. Those under restricted suckling were  $\geq 2$  years old with body weight of  $45.3 \pm 1.2$  kg and  $15.8 \pm 1$  days postpartum. The ewes in this group were allowed to interact with their lambs in the mornings (7–9 am) and evenings (5–7 pm) for periods of 15 min, the lambs were kept in a different pen for the rest of the day. The experiment began in December and lasted 35 days.

The rams ( $n = 8$ , crossbred Dorper  $\times$  Pelibuey  $\times$  Katahdin) were separated into four groups: experienced-I ( $n = 2$ , 71.5 and 77.3 kg body weight), experienced-II ( $n = 2$ , 65 and 69.5 kg), inexperienced-I ( $n = 2$ , 46 and 52.2 kg) and inexperienced-II ( $n = 2$ , 41.6 and 53.3 kg). The rams in the experienced groups were  $\geq 2$  years old with sexual experience; they had previously mated with ewes. The rams in the inexperienced groups were 10–12 months old with no sexual experience; they had never been exposed to females after weaning or before the study. Care of the animals used in the present study complied with Mexican animal welfare regulations.

### 2.2. Nutrition and feeding

The rams were kept in the same pen during the entire experiment. They were fed twice a day with a 1.6 kg of a

ration containing 69.6% wheat straw, 30% wheat, and 0.4% limestone. The empty and maiden ewes were fed twice a day with 1 and 0.77 kg of the ration offered to males. The ewes in the groups of continuous and restricted suckling were fed twice a day with a 1.8 kg of a ration containing 49.2% wheat straw, 30% wheat, 20% soybean meal, and 0.8% limestone. Feed was always provided after ram exposure to ewes. Water was provided ad libitum to all animals.

### 2.3. Response variables

The measured response variables were the time that each group of rams spent courting each group of ewes during the first 22 days of the experiment, the number of ewes from each group that showed estrous behavior during and after the 22 days of the experiment, duration of estrus, and size of the corpus luteum and the largest ovarian follicle.

The time (minutes) that each group of rams spent in courtship activities (chasing, nudging, attempt to mount, sniffing anogenital area and performing flehmen reaction) were visually recorded for each group of ewes according to the following management procedure. A male from each group was introduced to each group of ewes. When courtship ceased for one minute, a second male of each group was introduced to reinforce male to female stimulation, this allows us to record the time that rams were fully dedicated to courting ewes not to other activities, such as eating, exploring surroundings, or fighting. Time recording began when the first male of each group was introduced to each group of ewes and ended when both males of each group ceased courtship for one minute. To prevent copulation, the males always wore an apron during courtship. The ewes from each group were daily exposed at the same time to a different pair of males (experienced and inexperienced) at 10 to 12 h intervals for a period of 22 days. Therefore, within a period of two days, a pair of males was exposed to the four groups of ewes (maiden ewe lambs, empty ewes, ewes under continuous suckling and ewes under restricted suckling), and each pair of males were 11 times exposed to the same group of ewes within a 22-day period.

The number of ewes from each group that showed estrus during exposure to rams was recorded. An ewe was regarded in estrus when she stood to be mounted by a ram. Recording the duration of the estrus (h) began when the ewe was in estrus and ended when she no longer accepted to be mounted by a ram [21]. Both variables were measured at 12 h intervals over 35 days. The ewes showing estrus were temporarily removed to a different pen to prevent rams from fighting. All the ewes were injected i.m. with 12.5 mg of dinoprost (Lutalyse, Zoetis) in the afternoon of day 22 of the study. The number of ewes showing estrus from each group, and duration of estrus was measured for 13 days after prostaglandin injection. Both variables

were measured as previously indicated, using several rams wearing an apron.

The diameter (mm) of the largest ovarian follicle and corpus luteum was measured at the beginning of the study by trans-rectal ultrasonography (Aloka SSD-500, with 7.5 MHz linear transducer; Aloka Ltd, Tokyo, Japan). Diameter of both ovarian structures was calculated by averaging horizontal and vertical measurements. To avoid rectal injury and animal distress, ultrasonography was not performed on maiden ewes. Diameter of the corpus luteum was also measured before prostaglandin injection, but only in the ewes from continuous and restricted groups.

#### 2.4. Statistical analysis

The data from males in experienced-I and experienced-II were merged into a single group, the same process was carried out in the inexperienced groups, which yield two experimental groups (experience and inexperienced rams), each group with 88 observation (22 observations per group of ewes). The time that rams spent in courtship did not satisfy the homogeneity of variance and normality test. A logarithmic transformation was applied to the data, which was analyzed using a factorial design with two factors, ram sexual experience with two levels (inexperience vs experience), and reproductive status of ewes with four levels (maiden, empty, continuous and restricted suckling), in a completely random design. Means were compared with the Scott and Knott test [22].

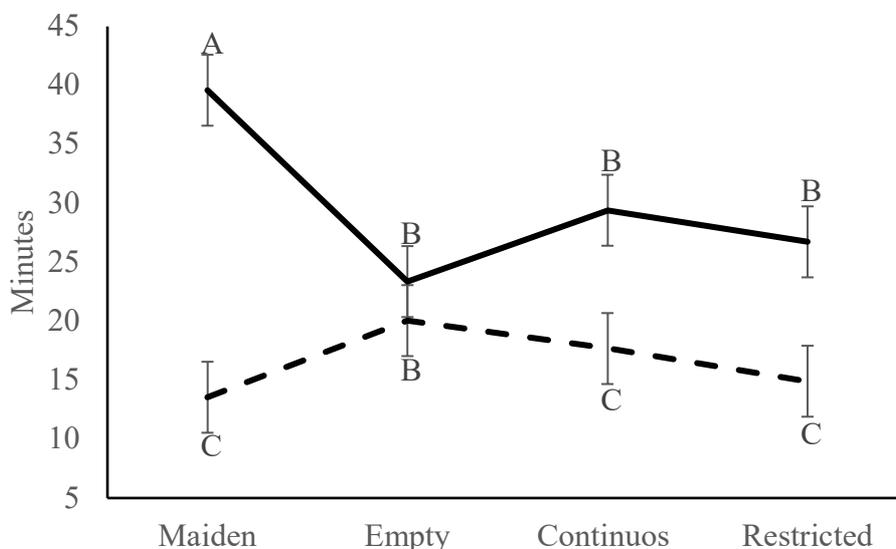
The percentage of ewes showing estrus in every group during the first 22 days of ram exposure and after prostaglandin injection was analyzed by Chi-square and McNemar tests.

The lack of information of the response variables duration of estrus and size of the corpus luteum and the largest ovarian follicle in the maiden group prevented us from analyzing this information under any experimental design. However, descriptive statistics of these variables are shown. The statistical package SPSS was used throughout the work.

#### 3. Results

The time that males dedicated to courtship was affected by the level of ram experience (inexperienced vs. experienced) and by the ewes' reproductive status (maiden, empty, restricted, and continuous suckling) and their interaction. Despite the interaction, overall, experienced rams spent less time courting ewes than inexperienced rams. Among the female groups, maiden and empty ewes were more attractive to inexperienced and experienced rams ( $p \leq 0.05$ , Figure).

The results regarding diameter of the largest ovarian follicle at the beginning of the experimental period, number of observed estrus, estrus duration, and number of ewes that displayed estrus are shown in Table. The percentage of ewes showing estrous behavior during the 22 days of ram exposure was affected by the reproductive status of the ewes; the largest ( $p \leq 0.05$ ) difference was observed in the empty group (Table). In addition, a higher number ( $p \leq 0.05$ ) of ewes was observed in estrus after prostaglandin injection (13 vs 22). The diameter of the corpus luteum was  $5.95 \pm 1.18$  mm in ewes from the empty group at day zero of the experiment, corpus luteum was not detected in any ewes from the continuous and restricted group at this



**Figure.** Time dedicated to courting ewes with different reproductive status by experienced (dashed line) and inexperienced (solid line) rams. Observations with different letter are significant different ( $p \leq 0.05$ ).

**Table.** Reproductive variables measured in ewes with different reproductive status during and after ram exposure.

Variable	Group			
	Empty	Continuous suckling	Restricted suckling	Maiden
n	10	10	10	10
Diameter of the largest follicle (mm) at day 0 <sup>§</sup>	3.96 ± 1.40	4.94 ± 1.02	4.96 ± 1.37	NR
Number of ewes with a corpus luteum at day 0	9	0	0	NR
Number of ewes with a corpus luteum at day 22	NR	5	8	NR
Number of observed estrus*	19	8	8	5
Estrus duration (h) <sup>*§</sup>	41.42 ± 16.16	17.68 ± 6.16	23.12 ± 9.15	22.8 ± 12.11
Number of ewes showing estrus until day 22	8	2	1	2
Number of ewes showing estrus after prostaglandin injection	9	5	7	1

NR: not recorded. <sup>§</sup> Means ± standard deviation. \* Number of times that estrus was observed during the entire experimental period (35 days). <sup>\*</sup> Estrus duration was calculated using data from estrus observed during the entire experimental period.

time. The diameter of the corpus luteum was  $9.15 \pm 2.89$  mm and  $8.27 \pm 1.50$  mm in ewes from the continuous and restricted groups at day 22 of the experimental period.

#### 4. Discussion

Age and experience shape the characteristic sexual behavior observed in sheep. In the present study, there is clear evidence that attractiveness of the ewes to rams depends on the level of sexual experience of the male and the female's reproductive status. Sexually experienced rams spend more time courting empty ewes. This implies that experienced rams might have the ability to differentiate between sexually available partners and those that are not ready for sexual interaction. In this case, the ewes in the empty group would be more sexually available partners. This is confirmed by the fact that this group displayed stronger estrous behavior than other groups of ewes.

There are several factors that modified the preference of ram to mate with a particular ewe [23], such factors include the scent [24], the nutritional status [25] and the physical appearance [12] of the ewe. Additionally, there is evidence that rams are more sexually attracted to older sexually experienced ewes than to naïve young ewes [5], which support the idea that rams have the ability to differentiate between sexually available partners because more ewes in the empty group were cycling than in the maiden group (Table). Such behavior does not seem to be present in young sexually inexperienced rams, because they dedicated more time to courting maiden ewes than other groups of ewes, a fact worth noting because the level of experience does not affect the endocrine response of rams to ewes in estrus [26]. Contrary to our results, rams that are more sexually interested in ewe lambs than in mature ewes have been reported [27]. A possible explanation for

this controversy might be that ewe lambs used in the latter study were heavier and probably closer to puberty than those used in our experiment. Those attributes could have made ewe lambs more attractive to rams.

The sexually inexperienced rams in this study spent more time courting maiden ewes than the rest of the groups, but the duration dedicated to courting empty ewes was similar to the one dedicated by experienced rams. The maiden ewes, based on incidence of estrus, can be disregarded as the least sexually available group. The present study does not have the necessary elements to explain this behavior in young rams. However, it is known that ewes prefer older larger rams over smaller younger rams [7]. Therefore, it is possible that ewes in groups other than maiden females had restricted voluntary courtship with inexperienced males. On the other hand, the sexual inexperience of maiden ewes made them unable to restrict courtship with young rams. Experience is important to fully develop characteristic sexual behavior and to properly respond to sexual stimulus [6, 28]. Accordingly, maiden ewes with previous interactions with rams showed more proceptive behavior to rams than naïve ewes [29]. In contrast, sexual inexperience made naïve ewes respond to male encounters stressfully [30] and with behavioral changes, such as running away from the ram. We know that running away from a male within a closed pen stimulates chasing in young rams. Thus, it is likely that the sexual inexperience of maiden ewes and young rams were additive effects to the time dedicated to courtship.

Expression of estrous behavior in sheep is down-regulated by long days of the season and lamb suckling after lambing, and it is absent in pre-pubertal females [31, 32]. The expression of estrus in the empty, continuous, and restricted suckling groups of ewes was as expected

during the first 22 days of the study. The higher incidence of estrus in empty ewes indicates that estrous cyclicity was established; this is because the study was carried out during the reproductive season of the year (December, short days). In contrast, the lower number of ewes showing estrus in the continuous and restricted groups indicates that most of the females were in postpartum anestrus, which is in part a result of lamb suckling during nursing.

The lamb suckling after lambing disturbs gonadotropin secretion [33 - 35] and delays the appearance of the first estrus and the establishment of estrous cyclicity in postpartum ewes [36]. Breaking anestrus in the continuous suckling postpartum ewes was attempted by exposing them to the “male effect” for 25 days [37]. Such an approach is acceptable since it is known that the “male effect” restores the neuroendocrine mechanism responsible for inducing ovulation in seasonal anestrus ewes [17]. However, expression of estrous behavior among ewes under continuous and restricted lamb suckling was scarce (2 / 10 and 1 / 10). On the other hand, after the first 22 days of the experiment, a higher number of corpora lutea was detected in the restricted and continuous lamb suckling groups, indicating that several ewes ovulated without showing signs of estrus (silent ovulation). Silent ovulation in postpartum ewes is

not rare [38]. Unfortunately, the experimental design of the present study did not allow us to determine whether silent ovulation occurred naturally or in response to the “male effect”, mainly because there is not a control group (without male stimulation) for each experimental group of ewes. In addition, a daily blood progesterone profile or periodically ovarian ultrasonography to detect a corpus luteum would be advisable to better identified the end of the postpartum anestrus.

The number of ewes showing estrous behavior increased after prostaglandin injection, mainly in the continuous (5 / 10) and restricted groups (7 / 10). Studies have shown that suckling restriction improves reproductive response after hormonal treatment in postpartum ewes [39], suggesting a positive effect of ram stimulation in continuous suckling ewes on estrous response after prostaglandin injection. In contrast, the poor incidence of estrus in maiden ewes after the prostaglandin injection (1 / 10) and during the 35 (3 / 10) days of the experiment clearly indicated that most of them had not yet reach sexual maturity.

## 5. Conclusion

In conclusion, the level of sexual experience and the reproductive status of the ewes affects the time that rams spend in courtship.

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