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Effects of ram presence during synchronization period and previous experience on certain estrus parameters and sexual behaviors in Kıvrıkcık ewes

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Abstract: This study aimed to investigate the effects of ram presence during estrus synchronization protocol and previous sexual experience on estrus onset, estrus duration, and sexual behaviors in Kıvrıkcık ewes. The ewes were divided into 2 groups according to the presence or absence of rams during the synchronization protocol. Ewes in each group were also divided into 3 subgroups according to their previous sexual experience. Ram presence during synchronization protocol did not affect estrus onset, estrus duration, mean attractivity, mean proceptivity, and mean receptivity. Nonexperienced ewes had longer estrus onset and shorter estrus duration than experienced ewes. Experienced ewes were more attractive and more receptive than the naive ones. It is concluded that although ram presence during estrus synchronization has no effect on sexual behaviors, previous sexual experience with males improves the expression of sexual behaviors in Kıvrıkcık ewes. Therefore, detection of estrus in maiden ewes should be performed more carefully in order to get high rates of flock fertility.

Key words: Ram presence, experience, proceptivity, receptivity, estrus onset

1. Introduction

The success of reproduction is important for the profitability of the farm and also for sustainability of sheep breeding, since the greatest part of the income in sheep farming is supplied through lamb production (1). For successful reproduction both females and males should display adequate levels of sexual behavior (2,3). In ewes, sexual behaviors are only expressed for a short period during the estrous cycle, around the time of ovulation (4). Sexual behavior of the ewes may be divided into 3 components: attractivity, proceptivity, and receptivity. Attractivity refers to the stimulus value of the ewe in evoking sexual responses in the ram. Proceptivity consists of the appetitive behaviors expressed by the ewe towards the ram and includes the activities that initiate and maintain the sexual interaction by the ewe. Receptivity is the consummatory phase of the sexual interaction and includes the behaviors performed by the ewe that are necessary for successful copulation and allow intravaginal ejaculation (5).

Several factors, such as temperament (6), previous experience (6–9), age (7,8), and the presence of rams (10,11), have been reported to affect attractivity, proceptivity, and receptivity. The effects of ram presence following estrus synchronization protocol on the estrus responses of ewes have been investigated extensively. However, research

investigating the effects of ram presence during estrus synchronization protocol is limited. The present study aimed to evaluate the effects of ram presence during the estrus synchronization period and previous sexual experience with rams on estrus onset, estrus duration, attractivity, proceptivity, and receptivity in Kıvrıkcık ewes synchronized during the breeding season.

2. Materials and methods

The study was carried out at the Research Farm of the İstanbul University Veterinary Faculty during the breeding season. A total of 58 Kıvrıkcık ewes and 12 adult Kıvrıkcık rams were used in the study. The ewes were divided into 2 treatment groups according to the absence of rams (NR, n = 31) and presence of rams (RP, n = 27). During estrus synchronization the NR group was isolated from rams, while the RP group was kept together with rams. Ewes in each treatment group were also divided into 3 subgroups according to their previous sexual experience: 1) nonexperienced (NE), including 1.5-year-old naive ewes that had never mated with a ram (n = 18, 9 NR and 9 RP); 2) experienced - I (Exp - I), including 2.5-year-old ewes that had mated and lambled only once (n = 20, 11 NR and 9 RP); 3) experienced - II (Exp - II), including 3.5-year-old multiparous ewes (n = 20, 11 NR and 9 RP). Estrus

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was synchronized with intravaginal sponges impregnated with 30 mg of fluorogestone acetate left for 12 days, plus an injection of 600 IU of pregnant mare's serum gonadotropin (PMSG) at the time of sponge removal.

Behavioral observations were performed at 16, 24, 32, 40, 48, 56, 64, and 72 h after sponge removal. In each observation period, ewes were introduced to rams one by one in the observation pen, and all sexual behaviors of the ewes and rams were video-recorded for 3 min. For each observation period, the sexual behaviors of each ewe were noted on individual check-sheets by watching the video records. The frequency of each behavior was determined for each ewe for each observation period. During the evaluation of video records, soliciting, scrotum sniffing, head-turning, anogenital sniffing, nonfirm-standing, squatting, tail-fanning, and following were recorded as proceptive behaviors, whereas firm-standing was recorded as receptive behavior (3,12).

A ewe receiving any courtship behavior by a ram, whether she responded or not, was accepted as attractive. The attractivity of each ewe for each observation period was calculated from the frequency of the ram's sexual behaviors, such as sniffing, nosing, nudging, kicking, and mounting. A ewe displaying proceptive behaviors, interested in the ram and accepting the courtship behaviors of the ram but not allowing mounting or mating, was considered proceptive. The proceptivity index (PI) of each ewe at each observation period was calculated as the percentage of permissions given by the ewe to the courtship attempts of the ram over the total number of courtship attempts by the ram in that observation period. A ewe standing still in response to the mounting attempts of the ram and allowing mounting or mating was assumed to be receptive. The receptivity index (RI) of each ewe at each observation period was calculated as the

percentage of immobilizations expressed by the ewe over the total number of mounting attempts by the ram. Mean attractivity and mean PI for each ewe were calculated by averaging the means of each characteristic determined at 8 observation periods, whereas mean RI was calculated by averaging the RI of observation periods in which the ewe was receptive.

The time period between sponge withdrawal and the midpoint of the time interval between the last rejection to be mounted and the first tolerance was assumed as estrus onset (3,12,13). The length of time between the onset of estrus and when a ewe no longer accept mounting by the ram was assumed as estrus duration (3,12).

To determine the effects of ram presence and ewe experience on estrus onset, estrus duration, mean attractivity, mean PI, mean RI, and PI and RI at different observation periods, least-squares procedures were performed using SPSS 10.0. The model of these characteristics included the fixed effects of ram presence (NR, RP), experience (NE, Exp - I, Exp - II), and ram presence \times experience interactions. Data for receptive ewe percentages at different observation periods were analyzed by the chi-square method.

3. Results

Ram presence during estrus synchronization has no effect on estrus onset, estrus duration, mean attractivity, mean PI, and mean RI (Table 1). On the other hand, previous sexual experience affected estrus onset, estrus duration, mean attractivity, and mean RI significantly ($P < 0.05$). NE ewes came into estrus later and had a shorter estrus duration than the Exp - I and Exp - II ewes ($P < 0.05$). Mean attractivity increased with previous sexual experience, and Exp - II ewes were more attractive than the naive ones ($P < 0.05$). Although the differences between groups were not

Table 1. Least squares means \pm standard error for estrus onset (h), estrus duration (h), mean attractivity, mean PI (%), and mean RI (%) in treatment (T) and ewe experience (E) groups.

Trait	Treatment (T)		Experience (E)			Significance (P-value)		
	NR	RP	NE	Exp - I	Exp - II	T	E	T \times E
Estrus onset (h)	35.19 \pm 1.19	33.04 \pm 1.27	37.78 ^a \pm 1.56	33.13 ^b \pm 1.48	31.43 ^b \pm 1.48	0.221	0.014	0.889
Estrus duration (h)	22.84 \pm 1.41	19.56 \pm 1.50	16.89 ^b \pm 1.84	24.12 ^a \pm 1.75	22.59 ^a \pm 1.75	0.116	0.017	0.525
Mean attractivity	8.22 \pm 0.19	8.27 \pm 0.20	7.74 ^b \pm 0.25	8.42 ^{ab} \pm 0.24	8.59 ^a \pm 0.24	0.850	0.042	0.990
Mean PI (%)	48.44 \pm 1.91	44.38 \pm 2.04	42.00 \pm 2.49	46.87 \pm 2.38	50.36 \pm 2.38	0.151	0.060	0.959
Mean RI (%)	68.54 \pm 3.49	68.32 \pm 3.72	58.42 ^b \pm 4.56	78.84 ^a \pm 4.35	73.02 ^a \pm 4.35	0.967	0.030	0.974

NR: No rams; RP: rams present. NE: nonexperienced; Exp - I: experienced - I; Exp - II: experienced - II. PI: Proceptivity index; RI: receptivity index.
^{a,b}: Differences between the means for experience groups with various letters in the same line are significant ($P < 0.05$).

significant statistically, mean PI tended to increase with experience ($P = 0.060$). NE ewes had lower mean RI than the experienced ones ($P < 0.05$).

The effects of ram presence and previous sexual experience on indices of proceptivity and receptivity at each observation period are presented in Figure 1. During the behavioral observations, proceptivity showed parallel changes in the NR and RP groups and the differences between groups were not significant, except at 56 h (Figure 1a). In both groups proceptivity started to increase at 16 h, reached a peak at 40–48 h following sponge removal, and then started to decline. Probably ewes in both groups continued to express proceptive behaviors after 72 h since it did not cease at 72 h. RI also showed similar changes in both groups (Figure 1b).

Time-dependent changes in proceptivity were similar in the NE, Exp - I, and Exp - II groups (Figure 1c). Experienced ewes tended to display proceptive behaviors a little more than the naive ones and the differences between groups (NE vs. Exp - II) were significant at 32 h. Although receptivity showed a similar pattern in all 3 groups, it was generally lower at all observation periods in the NE group than the experienced groups, and the differences between groups (NE vs. Exp - I and Exp - II) were significant at 32 h ($P < 0.05$) and 40 h ($P < 0.01$) (Figure 1d).

Ewes started to mate at 24 h in the NR group and at 32 h in the RP group, and matings accumulated in a shorter time period in the RP group (Table 2). No receptive ewe was observed after 56 h in the RP group and after 64 h in the NR group. Therefore, the percentage of ewes displaying receptive behaviors was significantly lower in the RP group than the NR group at 56 h ($P < 0.05$) and 64 h ($P < 0.01$). The differences in percentage of ewes expressing receptive behavior were not significant among experience groups (NE, Exp - I, Exp - II) except at 32 h (Table 2). While there was no receptive ewe at 24 h following sponge removal in the NE and Exp - II groups, 10% of ewes in the Exp - I group were receptive. In the 3 groups, no receptive ewe was observed after 64 h.

4. Discussion

The results of the current study indicate that ram presence during estrus synchronization in the breeding season has no effect on estrus onset and estrus duration in Kivırcık ewes. For successful fertilization, estimation of ovulation time, which is related to estrus onset, is important, especially in artificial insemination programs (14,15). Therefore, it is essential to determine the factors that affect estrus onset. In ewes, the time of ram introduction (early vs. late) and type of ram presence (continuous vs. intermittent) after estrus

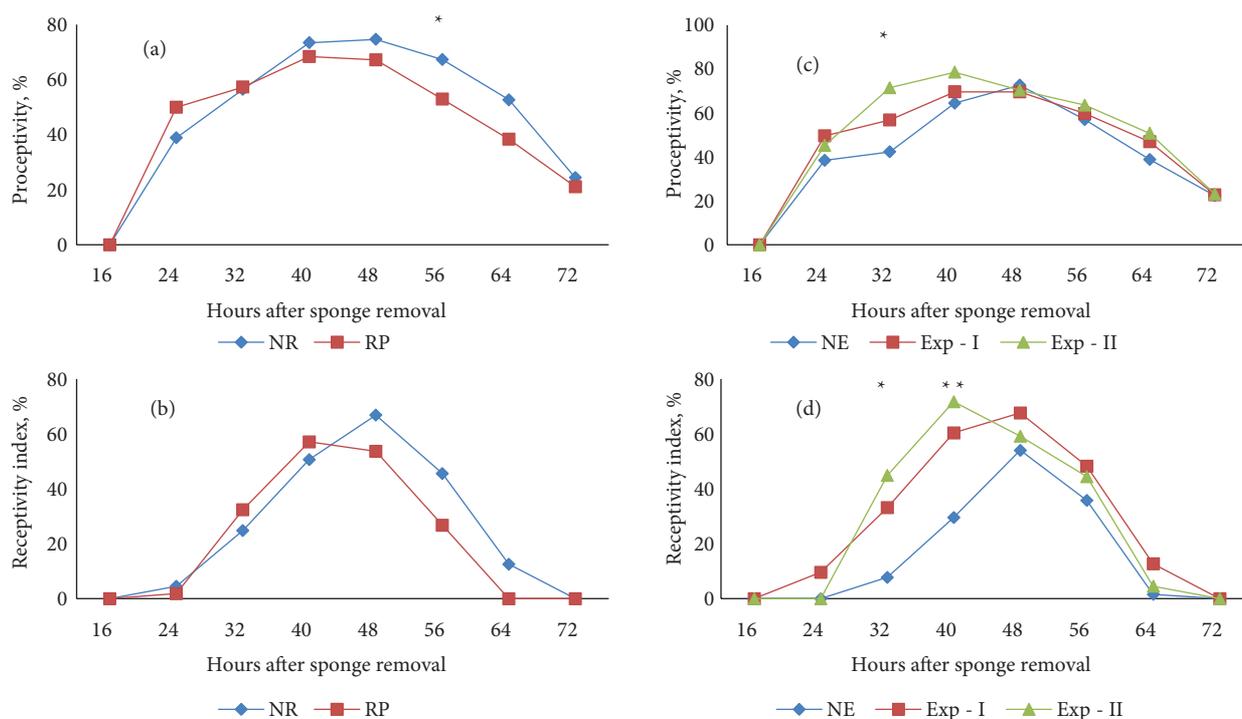


Figure 1. Changes in proceptivity after sponge removal in treatment (a) and experience (c) groups, and changes in receptivity after sponge removal in treatment (b) and experience (d) groups. * $P < 0.05$; ** $P < 0.01$.

Table 2. The percentages of ewes that expressed receptive behaviors after sponge removal.

Hour	Treatment effect			Experience effect			
	NR	RP	P value	NE	Exp - I	Exp - II	P-value
16	0	0	-	0	0	0	-
24	6.5	0	0.187	0	10.0	0	0.147
32	35.5	48.1	0.329	16.7 ^b	40.0 ^{ab}	65.0 ^a	0.010
40	71.0	85.2	0.195	61.1	80.0	90.0	0.098
48	93.5	77.8	0.082	88.9	90.0	80.0	0.607
56	61.3	29.6	0.016	38.9	60.0	40.0	0.329
64	22.6	0	0.008	5.6	20.0	10.0	0.371
72	0	0	-	0	0	0	-

^{a,b}: Differences between the percentages for experience groups with various letters in the same line are significant ($P < 0.05$).

synchronization affect estrus onset and estrus duration (3,14,15). However, research regarding exposure to, or isolation from, rams before estrus synchronization has had divergent results. It has previously been reported that an isolation period from males during the breeding season hastens and synchronizes estrus in ewes (10,13). However, recent studies indicate that an isolation period is not essential to hasten the onset of estrus or to get higher and more synchronized estrus responses from females (16–18). Supporting the current results, Cushwa et al. (16) observed similar estrus responses in ewes isolated from rams or kept together with rams before breeding. Ungerfeld et al. (17) also found no differences in estrus onset, the percentage of ewes in estrus, and time to luteinizing hormone surge of Corriedale ewes isolated from rams or kept close to the rams before estrus synchronization in the breeding season. Different results obtained from different studies could be explained by the physiological state of the ewes related to the time when the experiment was performed; namely, an external stimulus does not induce a greater response since ewes display their maximum reproductive capacity during the breeding season (10,17).

Experienced ewes came to heat earlier and had longer estrus duration than inexperienced ones (Table 1). Similar to the current results, Gelez et al. (7) also reported that young naive ewes became receptive later than the adult ones and tended to have shorter estrus duration. The shorter estrus duration observed in the NE group indicates that determination of receptive ewes should be performed more carefully in order to get a high rate of flock fertility, especially when maiden ewes are used for breeding.

For successful mating, both males and females should express adequate levels of sexual interaction. When a ram is introduced into a group of ewes, first he tries to find the estrous ones by sniffing the anogenital regions of the ewes. He then orients most of his courtship behaviors towards the receptive ewes (19). The behavior of the ewe plays an important role during this sexual interaction, and sexual behaviors such as tail-fanning, head-turning, soliciting, and standing still displayed by the ewe strengthen the response of the ram (19). In the current study, rams found the Exp - II ewes more attractive than the NE ones, and they displayed more courtship behavior towards the Exp - II ewes (Table 1). This can be explained by the level of sexual activity expressed by the ewes since proceptive behavior of the female is the most important cue for attracting the male (2). Supporting the current results, rams were also found to be less responsive to young maiden ewes than adult ones (7). The low level of attractivity observed in the NE group indicates that maiden ewes in the flock will be preferred by the rams less frequently; therefore, the chances of maiden ewes being mated will diminish, which, in turn, will result in a decreased fertility in the flock.

Proceptivity tended to increase with experience, although the difference among groups failed to reach significance ($P = 0.06$) (Table 1). Previous research (7,9) also showed that ewes without prior sexual experience display proceptive behaviors less so than the adult experienced ones. When the time-dependent changes in proceptivity are followed (Figure 1c), it is seen that the Exp - II ewes were more proceptive than the NE group at 32 h after sponge removal. This can be explained by the differences

observed in estrus onset. Experienced groups displayed more proceptive behaviors during the observation period at 32 h since their estrus onset coincided with this time period. On the other hand, NE ewes became receptive later than the experienced groups.

In this study, the Exp groups had a higher mean RI (Table 1) and were also more receptive at 32 h and 40 h (Figure 1d) than the NE group. Furthermore, the percentage of receptive ewes at 32 h was higher in the Exp - II group than the NE group (Table 2). These findings are consistent with previous suggestions that prior sexual experience with males improves the sexual behaviors of ewes (6,7,9). All of these results show that although the differences in proceptivity among groups did not reach significance, prior sexual experience plays an important

role in the acceptance of the ram for mating by the ewes.

It is concluded that although the presence of rams during estrus synchronization did not affect estrus onset, estrus duration, and sexual behaviors, it resulted in a more synchronized estrus response in Kivircik ewes. On the other hand, previous experience with rams shortened the onset of estrus and improved the attractivity of ewes and the expression of sexual behaviors. Therefore, detection of estrus in maiden ewes should be performed more carefully in order to get high rates of flock fertility.

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