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Sexual behavior and hormone levels of Kıvırcık ewes after estrus synchronization during and out of the breeding season*

Abstract

The aim of the study was to investigate the sexual behavior and hormonal changes in Kıvırcık ewes synchronized during and out of the breeding season. Feeding, individual and eliminative behaviors of the ewes were also recorded. Group I ewes were synchronized during the breeding season and Group II ewes were synchronized out of the breeding season. At the end of synchronization protocol, ewes were exposed to Kıvırcık rams (day 0, hour 0) and behavioral observations were started. Jugular blood samples were collected at the end of each observation period. Among sexual behaviors, head-turning and non-firm standing were displayed more frequently than soliciting, anogenital sniffing, sniffing scrotum and firm standing in both groups. Squatting and tail-fanning were not observed regularly. Defecation and sexual behaviors did not differ between groups. Group I displayed more lying ($P<0.001$), standing ($P<0.001$), and urination ($P<0.001$) whereas Group II displayed more eating ($P<0.001$), rumination ($P<0.001$), drinking ($P<0.01$) and walking ($P<0.001$). Estradiol levels around estrus did not differ between groups and showed a wave like pattern. While serum progesterone was at the basal level before and during estrus, it started to increase at 50 h and reached a level >0.5 ng/ml at 122 h in both groups. Estrus onset was 30.0±1.38 and 26.5±2.27 h, for Group I and II, respectively. Estrus duration was 19.0±2.20 and 18.0±2.86 h, for the same groups, respectively. In conclusion, Kıvırcık ewes synchronized during the anestrous period showed similar behavioral and hormonal responses, as in the breeding season.

Key Words: sheep, Kıvırcık ewe, sexual behavior, estrus synchronization, estradiol, progesterone

Zusammenfassung

Titel der Arbeit: **Sexualverhalten und Hormonspiegel nach Östrussynchronisation bei Kivircik Schafen innerhalb und ausserhalb der Zuchtsaison**

Untersucht werder das Sexualverhalten sowie Änderungen des Hormonspiegels bei Kivircikschafen nach einer Östrussynchronisation die inner- und ausserhalb der Zuchtsaison erfolgte. Fütterung und Verhalten der Tiere wurde ebenfalls aufgezeichnet. Die Muttern der Gruppe I wurden während der Zuchtsaison, die der Gruppe II ausserhalb der Saison synchronisiert. Nach der Synchronisation wurden die Tiere den Kivircikböcken (Tag 0, Stunde 0) zugeführt und mit den Verhaltensbeobachtungen begonnen. Nach jeder Beobachtungsperiode wurden Blutproben aus der *vena jugolaris* entnommen. In beiden Gruppen wurde bezüglich des Sexualverhaltens häufiger das Kopfwenden zum Bock und eine Verweigerung des Aufspringens beobachtet, als Paarungsbereitschaft, After- und Hodenschnüffeln und das sichere Stehen der Muttern. Die Neigung zum Harnlassen und wiederholte Schwanzbewegungen wurden nicht regelmäßig beobachtet. Beim Koten und dem Sexualverhalten unterschieden sich beide Gruppen nicht. Bei Gruppe I wurde mehr Zeiten für Liegen ($P<0,001$), Stehen ($P<0,001$) und Harnen, bei Gruppe II mehr für Fressen ($P<0,001$), Wiederkauen ($P<0,001$), Trinken ($P<0,01$) und mehr Bewegung beobachtet. Der Östradiolspiegel während des Östrus zeigte bei beiden Gruppen das gleiche Muster. Der Serumprogesteronspiegel zu Beginn und während des Östrus als Basiswert erhöhte sich nach 50 Stunden und erreichte nach 122 Stunden in beiden Gruppen Werte von >0,5 ng/ml. Der Östrusbeginn lag in Gruppe I bei 30,0 ± 1,38 bzw. 26,5 ± 2,27 Stunden bei Gruppe II. Die Östrusdauer betrug in den Gruppen I bzw. II 19,0 ± 2,20 bzw. 18,0 ± 2,86 Stunden. Zusammengefasst wird festgestellt, dass Kivircik Muttern nach Synchronisation außerhalb der Zuchtsaison sowohl beim Sexualverhalten als auch bei der Hormonreaktion ähnlich reagieren, wie bei einer Synchronisation innerhalb der Zuchtsaison.

Schlüsselwörter: Schafe, Kivircik, Sexualverhalten, Östrussynchronisation, Östradiol, Progesteron

* The present study was summarized from the PhD thesis of the first author.

Introduction

Reproduction plays an important role in animal breeding. The success of reproduction depends on some complex physiological events, which lead to the production and emission of mature gametes, and some behavioral changes that ensure ova and sperm will be in contact at the appropriate time (CARATY et al., 2002).

Sexual behaviors of the ewe could be classified as attractivity, proceptivity and receptivity, and all three categories of these behaviors are important for successful mating to occur (BEACH, 1976). Proceptivity consists of the behaviors performed by the female in response to stimuli received from males whereas; receptivity includes the behaviors exhibited by the female that allow intravaginal ejaculation. In ewes, proceptive and receptive behaviors are only expressed for a short period during the estrous cycle, around the time of ovulation (TILBROOK et al., 1990). The level of proceptive behavior clearly influences the chances of the ewe being mated, and the number of ewes that are mated will influence the fertility of the flock (TILBROOK et al., 1990). Therefore, it is important to have the knowledge of sexual behavior as it helps to improve fertility.

Ewes of most breeds are anestrous for some portion of the year and the seasonality of breeding activity in sheep limits the annual productivity of the ewe. Estrus synchronization is commonly used in order to induce estrus and ovulation during the anestrous period, and to synchronize the estrus and shorten the duration of lambings, thus minimizing labour costs, during the breeding season (WILDEUS, 1999; SCHNEIDER and REHBOCK, 2003).

In Marmara Region of Turkey, the greatest part of the income in sheep farming is supplied through lamb production and Kıvırcık breed is the most widespread breed of this Region. In Marmara Region, semi-intensive breeding is common, and the climatic and environmental conditions are convenient for out of season breeding. However, because of the cost of artificial insemination and lower level of fertility in practice, breeders mainly use natural mating. Therefore, the level of sexual behaviors expressed in synchronized estrus during the out of season breeding is as important as the sexual behaviors expressed during the breeding season. However, very few studies have been conducted examining the behavioral characteristics of Kıvırcık ewes synchronized out of the breeding season. The present study was undertaken to investigate the frequencies of sexual behaviors and hormonal changes in Kıvırcık ewes synchronized during and out of the breeding season. Individual, feeding and eliminative behaviors of the animals were also evaluated.

Materials and methods

The experiment was carried out in Istanbul University Faculty of Veterinary Medicine. Twenty-four mature multiparous Kıvırcık ewes, aged 3-4 years, and four Kıvırcık rams, aged 3-4 years, were used. The ewes were allocated into two groups of 12 ewes in each. Group I was synchronized in July (breeding season of the breed) and Group II was synchronized in December (out of breeding season). In each part of the study, the ewes were housed in two adjacent paddocks equipped with straw as bedding material, and 6 ewes were kept in each paddock. The ewes were given 500 g/day/ewe sheep pellets having 2400 Kcal ME and 13% crude protein throughout the experimental period. Grass hay and water were supplied ad libitum. Animal treatments and care

were approved by the Animal Care and Ethics Committee of Istanbul University Faculty of Veterinary Medicine.

Before the estrus synchronization protocol, each ewe was submitted to a general physical examination and to a vaginal observation. To identify the ewes in each paddock, numbers from one to six were painted on the hip region of the ewes. In each part of the study, estrus was synchronized by using intravaginal sponges impregnated with 30 mg of fluorgestone acetate (FGA; Chrono-Gest/Sponge, Intervet International B.V., Boxmeer, Holland) left for 14 days followed by an injection of 500 IU PMSG (Chrono-Gest/PMSG, Intervet International B.V., Boxmeer, Holland) and 5 mg of dinoprost (Dinolytic®, Pharmacia & Upjohn, Belgium) administered intramuscularly at the time of sponge removal (WILDEUS, 1999). One sexually experienced entire adult Kıvırcık ram was introduced into each paddock immediately after sponge withdrawal, and behavioral observations were started at 7.00 am (day 0, hour 0). The experimental schedule is summarized in Table 1.

Table 1
Experimental schedule (Experimenteller Ablauf)

Day	Treatment	
- 14	Sponge insertion	
0	Sponge removal PMSG and dinoprost i.m. Ram introduction at 7.00 am (day 0, hour 0)	
0-5	Behavioral observation	every day between 07.00 - 09.00 13.00 - 15.00 19.00 - 21.00 01.00 - 02.00
	Blood sample collection	at the end of each observation period

In each part of the study, behavioral observations were conducted by two trained observers every day from 7:00 to 9:00 am, from 13:00 to 15:00 pm, from 19:00 to 21:00 pm and from 1:00 to 2:00 am for five days following sponge withdrawal. The total observation time was 35 hours per group. The observers took their place at least 15 min before the beginning of each observation period. To eliminate the effect of observer, they changed their places after each observation period.

During the observations, soliciting, sniffing scrotum, head-turning, anogenital sniffing, non-firm standing, squatting and tail-fanning were recorded as proceptive behavior, and firm standing was recorded as receptive behavior. In addition to sexual behaviors, feeding (eating, rumination, drinking), individual (lying, standing, walking) and eliminative (urination, defecation) behaviors of the ewes were noted. All the behavioral traits investigated are described in Table 2. The behaviors of animals were noted on individual checksheets prepared for each ewe. Sexual behaviors were recorded when they were seen. Feeding, individual and eliminative behaviors were recorded in every 5 minutes using the time sampling technique (BOGNER, 1984).

Each ewe was considered to be in estrus when she was directly observed to accept a mount from the ram (ROMANO et al., 2000). Estrus onset was defined as the time elapsed between sponge removal and the middle of the time interval between the last rejection to be mounted and the first tolerance (UNGERFELD and RUBIANES, 1999; ROMANO et al., 2000). Estrus duration was defined as the time interval between the

onset of estrus and when a ewe no longer stood to be mounted (GODFREY et al., 2001). A ewe was considered to have responded to the treatment when she showed estrus during the observation periods (ROMANO et al., 2000). Ovulation response was determined according to the serum progesterone concentration and each ewe was considered to have ovulated when progesterone concentration was ≥0.5 ng/ml (PERKINS and FITZGERALD, 1994).

Table 2
Behavioral traits recorded during the observation periods (Verhaltensmerkmale während der Beobachtungszeiten)

Soliciting	The ewe approaches to the ram, nuzzles the body of him, shows a tendency to stay in the vicinity of the ram and follows him
Sniffing scrotum	The ewe sniffs the scrotum and anogenital region of the ram
Head-turning	The ewe stands and swings her head to look at the courting ram
Anogenital sniffing	The ewe allows the ram to sniff her tail and genitalia
Non-firm standing	The ewe stands in front of the ram in response to the courtship of him but does not allow him to mount and she avoids when the ram attempts to mount
Squatting	Typical urination posture of the ewe
Tail-fanning	Repeated movement of the tail
Firm standing	The ewe stands still to receive a mount attempt or mount
Eating	The ewe meets its feed intake
Rumination	Chewing the rumen content, which comes to the mouth
Drinking	The ewe meets its water need
Lying	Lying without showing any other behavior
Standing	Standing without showing any other behavior
Walking	Moving at a walk
Urination	Voiding urine
Defecation	Voiding faeces

Jugular vein blood samples (10 ml) were collected immediately after each observation period, from all ewes and allowed to cloth at room temperature. After centrifugation at 3500 rpm for 15 min, serum was harvested and stored at -20ºC for further analysis. Serum progesterone and estradiol concentrations were measured using a commercial radioimmunoassay kit (BioSource, Nivelles, Belgium). The sensitivities of the assays were 0.05 ng/ml for progesterone and 4.8 pg/ml for estradiol. The intra- and inter-assay coefficients of variation were 6.8 and 9.1% respectively for progesterone and 5.3 and 6.8% for estradiol. Serum progesterone concentrations were measured in the samples taken after 7:00-9:00 observation periods. Because the ewes in both groups did not display any sexual behavior after the 12th observation period, serum estradiol concentrations were measured in the samples collected after the first 15 observation periods of the study.

Percentage of ewes expressed proceptive and receptive behaviors; feeding, individual and eliminative behaviors were compared by Chi-square test. Estrus onset, estrus duration and serum concentrations of progesterone and estradiol were analyzed using independent samples t-test (SPSS, 1997).

Results

Frequency of proceptive behavior is presented in Figure 1. Frequency of proceptive behavior started to increase at 18 h in Group II, whereas it started to increase at 24 h in Group I (Figure 1).

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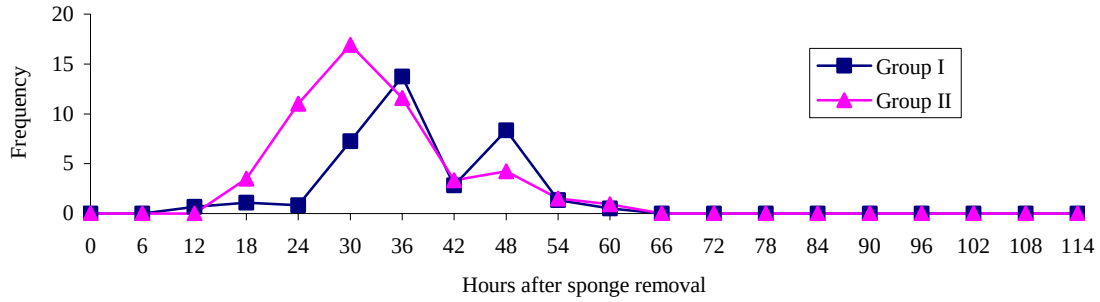

Fig. 1: Frequency of proceptive behavior after sponge removal (Häufigkeit des Abwehrverhaltens nach Synchronisation)

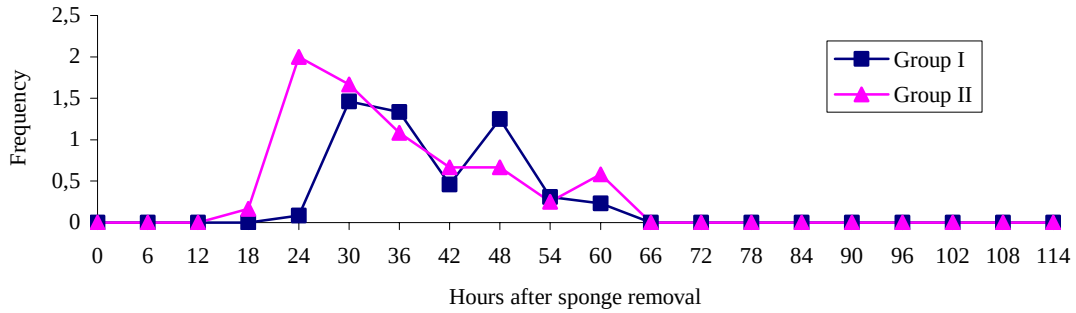

Fig. 2: Frequency of receptive behavior after sponge removal (Häufigkeit der Paarungsbereitschaft nach Synchronisation)

Table 3
The number (n) of Kıvırcık ewes expressed proceptive and receptive behaviors after sponge removal (Anzahl Kivircikmuttern mit oder ohne Paarungsbereitschaft nach Synchronisation)

Hour	Proceptive behavior		Receptive behavior	
	Group I (n=12)	Group II (n=12)	Group I (n=12)	Group II (n=12)
0	0	0	0	0
6	0	0	0	0
12	1	0	0	0
18	1	4	0	1
24	1**	7**	1	5
30	6	10	6	8
36	12	9	11	7
42	10	7	9	5
48	8	7	8	6
54	2	3	2	2
60	1	2	1	2
66-114	0	0	0	0

**: According to the results of Chi-Square test differences between the values differ significantly (P<0.01)

The number of ewes expressed proceptive and receptive behaviors are presented in Table 3. The differences between groups were not significant statistically except for proceptive behavior at 24 h. Frequency of receptive behavior is presented in Figure 2.

The differences between Group I and II for defecation and sexual behaviors were not significant (P>0.05). However, the differences between groups in respect of eating (P<0.001), rumination (P<0.001), drinking (P<0.01), lying (P<0.001), standing (P<0.001), walking (P<0.001) and urination (P<0.001) were significant (Table 4).

Table 4
Percentage (%) of feeding, individual, eliminative and sexual behaviors (Prozentualer Anteil beobachteter Verhaltensweisen)

Behaviors	Group I (n=12) %	Group II (n=12) %	Chi-Square
Eating	14.62	19.64	46.845***
Rumination	18.66	24.68	56.422***
Drinking	0.17	0.47	7.554**
Lying	24.03	19.94	25.757***
Standing	39.15	31.42	69.035***
Walking	1.25	2.14	12.554***
Urination	0.70	0.27	10.423***
Defecation	0.27	0.17	1.089
Sexual behaviors	1.16	1.27	0.285

** : Percentages in the same line differ significantly (P<0.01)
*** : Percentages in the same line differ significantly (P<0.001)

Changes in serum estradiol and progesterone levels are presented in Figures 3 and 4, respectively. Serum estradiol level did not differ between groups (P>0.05). The differences observed in serum progesterone concentration were not significant except at 48 h.

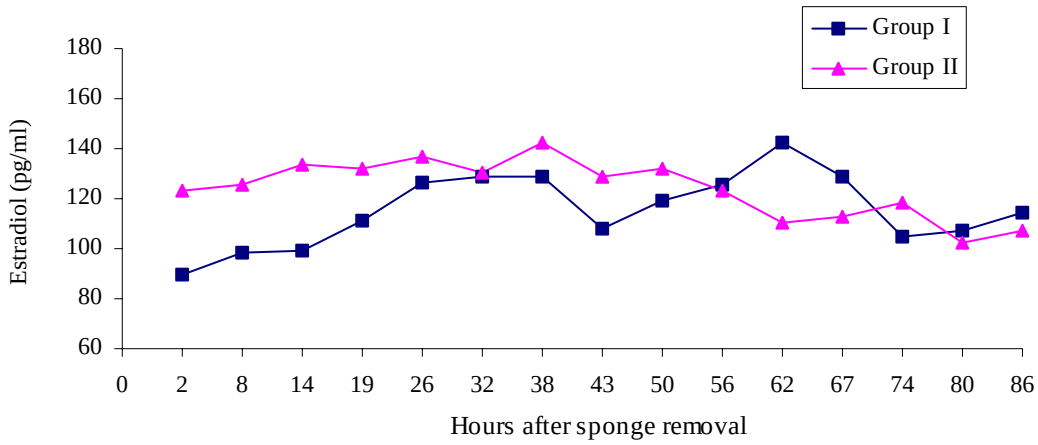

Fig. 3: Changes in serum estradiol level after sponge removal (Änderung des Östradiolspiegels nach Synchronisation)

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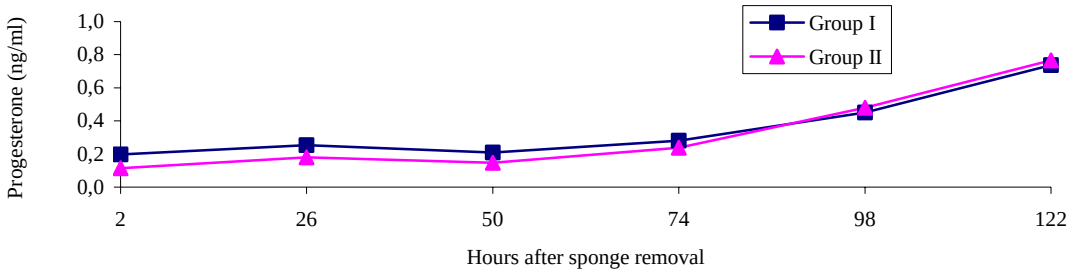

Fig. 4: Changes in serum progesterone level after sponge removal (Änderung des Serumprogesteronspiegels nach Synchronisation)

Estrus onset was 30.0±1.38 and 26.5±2.27 h for Group I and II, respectively (P>0.05). Estrus duration was 19.0±2.20 and 18.0±2.86 h, for the same groups, respectively (P>0.05) (Table 5).

Table 5
Means and standard errors (SE) of estrus onset and estrus duration (Mittelwert und Standardfehler von Östrusbeginn und –dauer)

Trait	Group I (n=12)		Group II (n=12)		t
	Mean	SE	Mean	SE	
Estrus onset (h)	30.00	1.38	26.50	2.27	1.316
Estrus duration (h)	19.00	2.20	18.00	2.86	0.227

Discussion

In the current study, ewes in both groups came on heat during the experimental period and ovulated in response to the treatment. The frequency of proceptive behavior and the number of ewes displaying proceptive behavior were highest at 36 h for Group I and at 30 h for Group II (Figure 1 and Table 3).Similar results were also reported for Préalpes (GELEZ et al., 2004) and Merinos (GELEZ et al., 2003) ewes. Among proceptive behaviors investigated in the present study, ewes in both groups displayed head-turning and non-firm standing more frequently than soliciting, anogenital sniffing and sniffing scrotum. However, tail-fanning and squatting were not observed regularly. EHNERT and MOBERG (1991) also reported that, although firm standing and looking-over-shoulder are associated with estrus, tail-fanning and squatting are not displayed consistently by Targhee ewes in heat. On the other hand, BRADLEY IMVALLE and KATZ (2004) suggested that tail wagging is the primary proceptive behavior expressed by female goats in estrus.

Ewes in Group I started to express receptive behavior at 24 h and in Group II at 18 h following sponge removal. This behavior was observed more frequently between 30-48 h in Group I and between 24-36 h in Group II (Figure 2). Number of ewes displaying receptive behavior did not differ significantly between groups and it was highest at 36 h for Group I and at 30 h for Group II (Table 3). In both groups the ewes did not express receptive behavior after 60 h following sponge removal. Similarly, CARATY et al. (2002) reported that, Ile de France ewes started to mate at 24 h following synchronization, and the matings started to decrease after 44 h and the ewes did not mate any more after 56 h. Similar to the current study, GELEZ et al. (2004),

who studied with Préalpes ewes, reported that all the ewes displayed receptive behavior on day 1 of estrus. However, 60% of these ewes also mated on day 2 of the estrus.

Among other behaviors, standing was the most frequently displayed behavior in both groups (Table 4). Although the percentage of standing was higher than those of KARAAĞAÇ et al. (2005), percentages of eating and lying were less than those of some other researchers (KUTSAROV et al., 2004; KARAAĞAÇ et al., 2005). The reason of this might be explained by being the ewes in estrus during the observation period. While the differences between groups for defecation and sexual behaviors were not significant; those for urination, individual and feeding behaviors were significant (Table 4). The significant differences between groups for these traits could be due to the difference in environmental temperature between July and December. SEVI et al. (2001) also reported that when the environmental temperature decreases the animals increase their food intake and physical activities, and vice versa.

Estradiol levels of Group I and II at 2 h following sponge removal were 89.98±6.81 and 123.60±19.54 pg/ml, respectively. It increased till 38 h and then showed a wave-like pattern (Figure 3). The changes in serum estradiol found in the current study are in accordance with the changes reported in previous studies (HOROZ et al., 1999; ZIEBA et al., 2001; BARRET et al., 2002; MENEGATOS et al., 2003). Estradiol level started to increase one day before the onset of behavioral estrus and it was high throughout the estrus. Peak estradiol level determined in the present study, was lower than that of Chois ewes (HOROZ et al., 1999) and higher than those of Western White-Faced (BARRET et al., 2002) and Chois (MENEGATOS et al., 2003) ewes. The differences determined in estradiol levels among studies might be due to the breed of ewe, or type and application time of gonadotrophine used for synchronization (BARTLEWSKI et al., 1999).

The changes in serum progesterone levels of both groups were similar to each other (Figure 4). At 2 h following sponge removal, progesterone level of Group I and II were 0.19±0.04 and 0.12±0.01 ng/ml, respectively. These levels were lower than 0.2 ng/ml, which was reported to be the basal level for ewes (BARTLEWSKI et al., 1999; BARRET et al., 2002). Similar to the reports of HOROZ et al. (1999) and MENEGATOS et al. (2003), progesterone levels of both groups were low before and throughout the estrus. Furthermore, LEYVA et al. (1998) reported that, there is a positive correlation between progesteron level and the diameter of the corpus luteum, and therefore progesterone level is low at the beginning of estrus since the corpus luteum has not been formed yet. In the current study, progesterone level was low till 50 h following sponge removal, then it started to increase and reached a level >0.5 ng/ml at 122 h in both groups.

The effects of season on estrus onset and estrus duration were found not to be significant (Table 5). It has been reported that (ROMANO et al., 2000; ROMANO et al., 2001; MENEGATOS et al., 2003; EVANS et al., 2004), some factors such as type of progestagen, use of PMSG and ram introduction might influence the estrus onset. The mean duration of estrus onset for both groups were within the range reported in previous studies (ROMANO et al., 2000; FUENTES et al., 2001; ROMANO et al., 2001; EVANS et al., 2004). However, there are some other studies (UNGERFELD and RUBIANES, 1999; GODFREY et al., 2001; BARRET et al., 2002; MENEGATOS et al., 2003) in which a longer interval to estrus has been reported than

that found in the present study. Estrus duration of Kıvırcık ewes determined in the current study was similar to those of St. Croix White (GODFREY et al., 2001), whereas it was shorter than those of Corriedale (ROMANO et al., 2001), Suffolk crosses (EVANS et al., 2004) and Suffolk X Hampshire crosses (FUENTES et al., 2001) ewes.

It is concluded that, Kıvırcık ewes synchronized during the anoestrous period show similar behavioral and hormonal responses as in the breeding season, and they display head-turning and non-firm standing more frequently than soliciting, anogenital sniffing, sniffing scrotum and firm standing when they are in estrous. However, they do not display squatting and tail-fanning regularly.

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