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Original Research Article

Effect of GnRH Injection on Fertility Parameters in Morkaraman Sheep in the Breeding Season

Key words

breeding beason;
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GnRH;
sheep;
synchronization

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Abstract: This study aimed to investigate the effect of GnRH injection on the first synchronization day on lambing performance and fertility in a short-term progesterone-based synchronization protocol in Morkaraman sheep during the breeding season. The study material consisted of 76 Morkaraman sheep in the breeding season. Clinically healthy and non-lactating ewes with an average age of 2-3 years, a BCS of 3.16±0.04, a weight of 63.98±0.79 kg, and five healthy fertile rams of adult age were included in the study. The rams were separated from the herd one month before the study started. The sheep were divided into two groups without intravaginal sponge placement. Intravaginal sponges (20 mg flugestone acetate, Chronogest®, France) were placed in all sheep to remain in the vagina for 6 days, and 1.5 mL $PGF_{2\alpha}$ (5 mg, Dinoprost, Enzaprost®, France) was injected intramuscularly 1 day before (day 5) and on the day the sponge (day 6) was removed. In the first group of sheep (n=39), 2 mL of GnRH (0.004 mg buserelin, Receptal®, Germany) was injected intramuscularly immediately after the sponge was placed in the vagina (Day 0). Unlike the first group, sheep in Group II (n=37) were injected with physiological saline (2 mL, i.m.) after the sponge was placed intravaginally. Immediately after the sponge was removed (day 6), all sheep were injected with 600 IU of eCG (Chrono-gest/PMSG, Germany), and the rams joined the herd. After mating the ram, the oestrus was monitored for 5 days. Pregnancy examinations were performed transrectally on the 30th day following mating. It was determined that the vaginal sponge was lost in two sheep each in Groups I and II, and these sheep were excluded from the study. It was determined that 54.05% of the ewes in Group I and 48.57% of the ewes in Group II were pregnant. Lambing was observed in all pregnant ewes. The multiple pregnancy rate was found to be less in Group I (45%) than in Group II (52.94%). A total of 32 lambs were obtained in Group I, and 26 lambs were obtained in Group II. Average lamb weights were found to be similar in both groups. As a result, GnRH injection combined with intravaginal sponge application may contribute to fertility success by numerically increasing the rate of estrus, pregnancy, and litter size.

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Introduction

Many different synchronization protocols are applied to increase productivity in sheep with seasonal polyestrus. The most preferred hormone for synchronization is progesterone (1). Progesterone controls sexual cycles by imitating the corpus luteum (2–5). Progesterone-impregnated sponges are usually left in the vagina for 12-19 days (6). Since long-term progesterone use may have a suppressive effect on fertility in subsequent cycles, short-term applications have been preferred in recent years (2–5). In addition, short-term progesterone treatment provides a high

fertility rate because it ensures exposure to high progesterone concentrations throughout the treatment period (7). It is reported that there are high success rates in short-term progesterone treatments of 5-6 days (7–9).

Gonadotropin-releasing hormone (GnRH) is a neuropeptide that plays a key role in the control of reproductive function. This hormone stimulates the preovulatory secretion of luteinizing hormone (LH) from the anterior pituitary gland (8). An LH peak occurs within 1-4 hours following GnRH treatment (10, 11). Increasing LH concentration stimulates ovulation, luteinization, and follicular atresia (12–14). GnRH does not exert its effect only via LH. In the absence of inhibin and estrogen, GnRH injection also stimulates follicle-stimulating hormone (FSH) production (15). A single dose of GnRH injection is used for the development of ovarian follicles in cows (16). The number of studies in which GnRH injection was performed on the day progesterone-based synchronization protocols started in sheep is quite low (5, 14). When GnRH is given before a short-term progestagen treatment, a new wave of follicular growth is initiated (17, 18), the number of follicles synchronized with ovulation time will increase (5), estrus, ovulation (17), and the lambing rate will increase, thus contributing to the increase in offspring yield (5). The response to GnRH depends on the phase of the estrous cycle, and sometimes there may be no response (19, 20). Progesterone prevents the subsequent spontaneous ovulation of dominant follicles that do not respond to GnRH injection, thus ensuring better synchronization (21, 22). If a luteal tissue is formed due to GnRH injection, the injected $PGF_{2\alpha}$ can cause the lysis of that luteal tissue (22, 23). Both removal of the vaginal sponge and stimulation of luteolysis by $PGF_{2\alpha}$ injection allow for a sudden drop in progesterone, which will increase follicular development and estrus formation (22).

The presented study aimed to investigate the effect of GnRH injection on the first day of synchronization on lambing performance and fertility in a short-term progesterone-based synchronization protocol in Morkaraman sheep during the breeding season.

Material and methods

Animal material

The presented research was carried out on 76 Morkaraman sheep during the breeding season, raised at Iğdır University Application and Research Farm, located at 39° north latitude and 44° east longitude. In the breeding season in 2022, clinically healthy and non-lactating ewes with an average age of 2-3 years old, with a BCS of 3.16±0.04 and a weight of 63.98±0.79 kg, and 5 healthy fertile rams aged 3-4 years were included. Sheep fed in the pasture were fed 500 g of barley and 300 g of meadow grass. Starting two weeks before the ram was introduced, 1.5-2 kg of barley, clover, and meadow grass mixture was given to the rams for flushing purposes. Water was given ad libitum.

Methods

The rams were separated from the herd one month before the study started. Intravaginal sponges (20 mg flugestone acetate, Chronogest®, France) were placed in all sheep to remain in the vagina for 6 days, and 1.5 mL $PGF_{2\alpha}$ (5 mg, Dinoprost, Enzaprost®, France) was injected intramuscularly 1 day before (day 5) and on the day the sponge (day 6) was removed. The sheep were divided into two groups just before the intravaginal sponge was placed. In the first group of sheep (n=39), 2 mL of GnRH (0.004 mg buserelin, Receptal®, Germany) was injected intramuscularly immediately after the sponge was placed in the vagina (Day 0). Unlike the first group, sheep in Group II (n=37) were injected with physiological saline (2 mL, i.m.) after the sponge was placed intravaginally. Immediately after the sponge was removed (day 6), all sheep were injected with 600 IU eCG (Chrono-gest/PMSG, Germany), and the rams joined the herd (Figure 1).

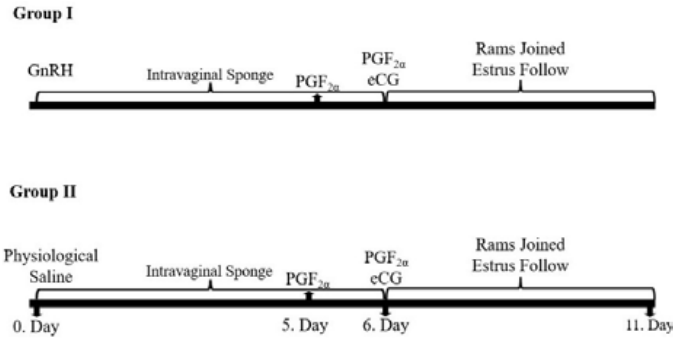

Figure 1: Synchronization protocol in groups

After the rams joined the herd, the estrus was monitored 3 times a day (8 hours apart) for 30 minutes for 5 days. The dates and times of estrus were recorded. On the 30th day following mating, pregnancy examinations were performed transrectally by the same veterinarian using a B-mode ultrasonography (7.5 mHz, Hasvet model 838, HASVET, Turkey) device. Lambing time, gender, and weight of the offspring were recorded.

Reproductive parameters were calculated using the following formulas using the data obtained from the study:

- Estrus rate (Number of ewes showing estrus/Total number of ewes)x100,
- Pregnancy rate (Number of pregnant ewes/Total number of ewes)x100,

- Lambing rate (Number of lambing ewes/Number of pregnant ewes)x100,
- Fertility (Number of ewes lambing/Number of ewes mating)x100,
- Lambing of a single offspring (Number of ewes lambing with one lamb/Number of ewes lambing)x100,
- Multiple lambing (Number of ewes giving birth with two or more lambs/Number of ewes lambing)x100
- Fekunditiy (Number of lambs born/Number of ewes mated),
- Litter size (Number of lambs born/Number of ewes lambing).

Obtaining blood serums and progesterone analysis

Blood samples were collected from the jugular vein just before the vaginal sponge was inserted (day 0), on the 5th day of the sponge, on the day the sponge was removed (6th day), and at the moment of detection of estrus. The collected blood was centrifuged (3500 rpm, 10 minutes), and serum was removed. Serums were stored at -20 °C until measurements were made. Progesterone level was analyzed by the ELISA (BT LAB, China, sensitivity: 0.097 ng/mL, detection range: 0.2-60 ng/mL) method.

Statistical analysis

Data analysis was performed using the IBM SPSS Statistics 25 software package. All data are presented as mean±standard error (mean±SE). Percentages between groups were compared using the Chi-square method. The Shapiro-Wilk test was used for normality tests of the data. Progesterone levels in the groups were compared with the Mann-Whitney U test. Student's T test were performed for the weights of the offspring. $P<0.05$ was considered significant.

Results

The average body weight of sheep in Group I was determined to be 66.47±1.08 kg, and in Group II was 61.37±1.01 kg. It was determined that the vaginal sponge was lost in two sheep each in Group I and Group 2, and these sheep were excluded from the study. When the vaginal sponge was removed, mild vaginitis was detected in 24 sheep in Group I and 15 sheep in Group II. Sheep that developed vaginitis were not excluded from the study. Reproductive parameters in the groups are presented in Table 1. It was determined that 54.05% of the ewes in Group I and 48.57% of the ewes in Group II were pregnant. Lambing was observed in all pregnant ewes. The multiple pregnancy rate was found to be higher in Group II (52.94%) than in Group I (45%).

Table 1: Reproductive parameters in groups

Parameters	Group I	Group II
Ewes joined (n)	37	35
Estrus rate (%)	30/37 (81.08)	24/35 (68.57)
Pregnancy rate (%)	20/37 (54.05)	17/35 (48.57)
Lambing rate (%)	20/20 (100.00)	17/17 (100.00)
Single lambing rate (%)	11/20 (55.00)	8/17 (47.06)
Multiple lambing rate (%)	9/20 (45.00)	9/17 (52.94)
Fecundity	32/30 (1.07)	26/24 (1.08)
Litter size	32/20 (1.60)	26/17 (1.53)

n= number of animal

The numbers of offspring born are presented in Table 2. While there were 11 singletons, 7 twins, 1 triplet, and 1 quadruplet lamb in Group I, it was determined that 8 singletons and 9 twin lambs were born in Group II.

Table 2: Distribution of the number of offspring born in groups

Parameters	Group I	Group II
Number of lambs born	32	26
Single lambing rate	11/20 (55.00)	8/17 (47.06)
Twine lambing rate	7/20 (35.00)	9/17 (52.94)
Triple lambing rate	1/20 (5.00)	-
Quadruplet lambing rate	1/20 (5.00)	-
Quintuple lambing rate	-	-
Six lambing rate	-	-

The average weights of newborn lambs are presented in Table 3. Average lamb weights were found to be similar in both groups.

Based on serum progesterone level results on the day synchronization started, it was determined that 21 of the sheep in Group I (21/37, 56.76%) and 31 of the sheep in Group II (31/35, 88.57%) had an active corpus luteum. On the day the study started, the average progesterone level in Group

Table 3: The average weight of offspring in groups

Groups	Average lamb weight (kg)	Average female lamb weight (kg)	Average male lamb weight (kg)
Group I	3.96±0.13	3.76±0.18	4.14±0.30
Group II	4.03±0.13	4.26±0.19	3.89±0.18

I was 1.04±0.07 ng/mL, while it was 2.63±0.33 ng/mL in Group II (P<0.001). No significant difference was detected between the groups in terms of serum progesterone levels on the other measurement days (Table 4).

Table 4: Serum progesterone level just before insertion of the vaginal sponge (day 0), on the 5th day of the sponge, on the day the sponge was removed (day 6), and on the day of estrus

	Progesterone (ng/mL)			
	0. day	5. day	6. day	Estrus
Group I (n=11)	1.04±0.07[a]	2.10±0.16	2, 53±0.25	0.57±0.03
Group II (n=12)	2.63±0.33[b]	2, 41±0.27	2, 36±0.21	0.84±0.02

a:b: <0,001; n= number of animals

Discussion

The presented study was planned with the expectation that GnRH injection administered simultaneously with progestagen administration in sheep during the breeding season would stimulate ovulation and luteinization of existing dominant follicles and contribute positively to fertility by stimulating a new follicle wave. It has been reported that more than half of the sheep (59%) had an active corpus luteum on the day the synchronization protocol was started (22) and that GnRH injection at the beginning of progesterone treatment would induce ovulation in more than 40% of the sheep (17). In the presented study, by looking at serum progesterone levels on the day the study started, it was determined that approximately 56.76% (21/37) of the sheep in the first group and 88.57% (31/35) of the sheep in the second group had an active luteal structure. 5 days after sponge insertion (just before $PGF_{2\alpha}$ injection), it was determined that the progesterone level in 3 sheep in Group I was still <1ng/mL. It was determined that the progesterone level in the remaining 13 sheep increased above 1ng/mL. The progesterone level on the 5th day in all sheep in Group II was >1ng/mL. It is thought that this increase in progesterone level on the 5th day in Group I is due to the stimulation of ovulations with the support of GnRH. Because synthetic progesterones cannot be measured in blood (24). Chronogest CR, which contains synthetic progesterone, has no relation to the increase in progesterone level on the 5th day.

There are various synchronization protocols accompanied by GnRH injections in sheep. One of these studies was by Martinez et al., in 2015 (17). In this study, a GnRH injection was administered to sheep in the breeding season immediately after CIDR was administered intravaginally. On day 7, CIDR was removed, and eCG and $PGF_{2\alpha}$ injection (U-synch) were performed. It has been reported that the U-synch protocol provides higher estrus, twinning, and a more acceptable fertility rate than traditional long-term progesterone treatment. According to Titi et al. (22), a GnRH injection was performed immediately after inserting a vaginal sponge into Awassi sheep during the breeding season. As a result of the treatment, the lambing rate was determined to be 47%, and the litter size was 1.4±0.3. It has been determined that there is an increase in lambing and fertility in animals administered GnRH + progesterone + $PGF_{2\alpha}$. Similarly, Karaca et al. (5) found in their study that GnRH injection administered on the day the short-term synchronization protocol was started significantly increased the multiple pregnancy rate and litter size (p<0.05). It is stated that these increases may be related to the growth of a new follicular wave in the ovary (5), following the increase in FSH and LH levels after GnRH injection, and the stimulation of synchronized follicle development and corpus luteum function (22, 25). It has been reported that if follicular dynamics are not controlled with GnRH injection before starting progestin treatment, low levels of constant release of progesterone may result in decreased fertility as a result of ovarian follicles reaching larger than normal sizes and persisting (26, 27).

In addition to these studies stating that GnRH injection applied on the first day of the synchronization protocol in sheep stimulates follicular development and is effective in estrus synchronization, other studies are reporting that it has no effect (28) or causes a negative effect (15, 29). Çizmeci et al. (14) administered GnRH injections on the first day of 9-day progesterone treatment in Akkaraman sheep outside the breeding season. It was found that estrus and pregnancy rates were lower than those without treatment. According to Santos-Jimenes et al. (28), GnRH injection was made immediately before intravaginal placement of the progesterone source, and the other group was not injected. Estrus and ovulation rates were determined to be 34.8% and 82.6% in the GnRH group and 48% and 96% in the non-GnRH injected group, respectively. In other words, it was observed that GnRH injection did not have a significant effect on fertility, and similar results were obtained in the group where GnRH was not administered. Since these studies were conducted outside the breeding season, no response to GnRH could be obtained. The presence of follicles on the ovary that will respond to GnRH is very important in the response to be received. In the absence of ovarian cyclicity, GnRH is not sufficient to generate LH release that triggers ovulation and luteinization (14).

Martinez-Ros et al. (15) determined that the time to emergence of estrus, preovulatory LH surge, and ovulation time as a result of GnRH injection, while the progesterone source

was placed in the vagina, were longer than in sheep that did not receive the progesterone source. This delay in preovulatory events is thought to be due to the change in follicular dynamics caused by GnRH injection. Similarly, another study revealed that the onset of estrus time in goats injected with GnRH on the day of short-term (8-day) sponge application was longer than in the group not injected with GnRH (29). It is reported that GnRH does not positively affect fertility parameters on the day of synchronization and causes a decrease in pregnancy and litter size rates. Although it is thought that GnRH injection on the day of sponge application may cause follicular cysts, it is stated that the exact cause has not been proven. In the presented study, it was found that the estrus rate in the GnRH group (81.08%) was higher than the other group (68.57%) (P>0.05). Although the pregnancy rate was numerically higher in the GnRH injection group, there was no statistically significant difference. However, the multiple pregnancy rate was found to be lower in the GnRH group than in the other group. However, the number of offspring per sheep was higher in the GnRH injection group. It is thought that the lack of a statistically significant difference between the groups may be due to the small number of animals in the study.

As a result, it was observed that GnRH injection combined with sponge application did not significantly affect some fertility parameters. In addition, the disadvantages of GnRH injection are that it requires extra labor and causes hormone costs.

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Ethical Approval. The present study was approved by the Animal Research Ethics Committee of the University of Kafkas (Ethics approval number: KAÜ-HADYEK, number 2022/211).

Declaration of interest. The authors have no conflict of interest to disclose.

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Vpliv aplikacije GnRH na parametre plodnosti pri ovcah pasme morkaraman v paritveni sezoni

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Izvleček: Namen raziskave je bil preučiti učinek odmerka GnRH prvi dan sinhronizacije na uspešnost jagnjitev in plodnost pri kratkoročnem protokolu sinhronizacije na osnovi progesterona pri ovcah pasme morkaraman med paritveno sezono. Raziskava je bila izvedena na 76 ovcah te pasme v paritveni sezoni. V raziskavo so bile vključene klinično zdrave ovce brez laktacije povprečne starosti 2–3 leta z oceno telesne kondicije 3,16 ± 0,04 in telesno maso 63,98 ± 0,79 kg ter pet zdravih odraslih plodnih ovnov. Ovni so bili ločeni od črede en mesec pred začetkom raziskave. Ovce so bile razdeljene v dve skupini pred vstavitvijo intravaginalnih gobic. Vsem ovcam so bile vstavljene intravaginalne gobice (20 mg flugeston acetata, Chronogest®, Francija), ki so ostale v nožnici 6 dni, en dan pred odstranitvijo gobice (5. dan) in na dan odstranitve (6. dan) so intramuskularno aplicirali 1,5 mL PGF2α (5 mg, Dinoprost, Enzaprost®, Francija). Prvi skupini ovc (n = 39) so intramuskularno aplicirali 2 mL GnRH (0,004 mg buserelina, Receptal®, Nemčija) takoj po vstavitvi gobice v nožnico (dan 0). Za razliko od prve skupine so ovcam v drugi skupini (n = 37) po intravaginalni vstavitvi gobice aplicirali fiziološko raztopino (2 mL, i. m.). Takoj po odstranitvi gobice (6. dan) so vsem ovcam aplicirali 600 IU eCG (Chrono-gest/PMSG, Nemčija), ovni pa so se pridružili čredi. Po pripustu ovna so estrus spremljali 5 dni. Preglede brejosti so opravili transrektalno 30. dan po parjenju. Ugotovljeno je bilo, da sta po dve ovci iz prve in druge skupine izgubili vaginalno gobico, zato sta bili izključeni iz raziskave. Brejost je bila ugotovljena pri 54,05 odstotka ovc iz prve skupine in 48,57 odstotka ovc iz druge skupine. Vse breje ovce so jagnjile. Ugotovljeno je bilo, da je bila stopnja večplodnih brejosti v prvi skupini (45-odstotna) manjša kot v drugi skupini (52,94-odstotna). V prvi skupini je bilo skupno 32 jagnjet, v drugi skupini pa 26. Povprečna teža jagnjet je bila v obeh skupinah podobna. Odmerek GnRH v kombinaciji z uporabo intravaginalne gobice lahko prispeva k uspešnosti plodnosti, saj številčno poveča stopnjo estrusa, brejosti in velikosti mladičev.

Ključne besede: : paritvena sezona; plodnost; GnRH; ovce; sinhronizacija